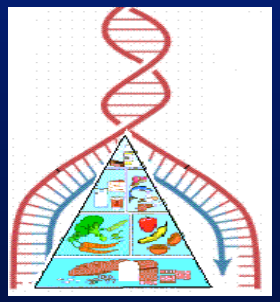
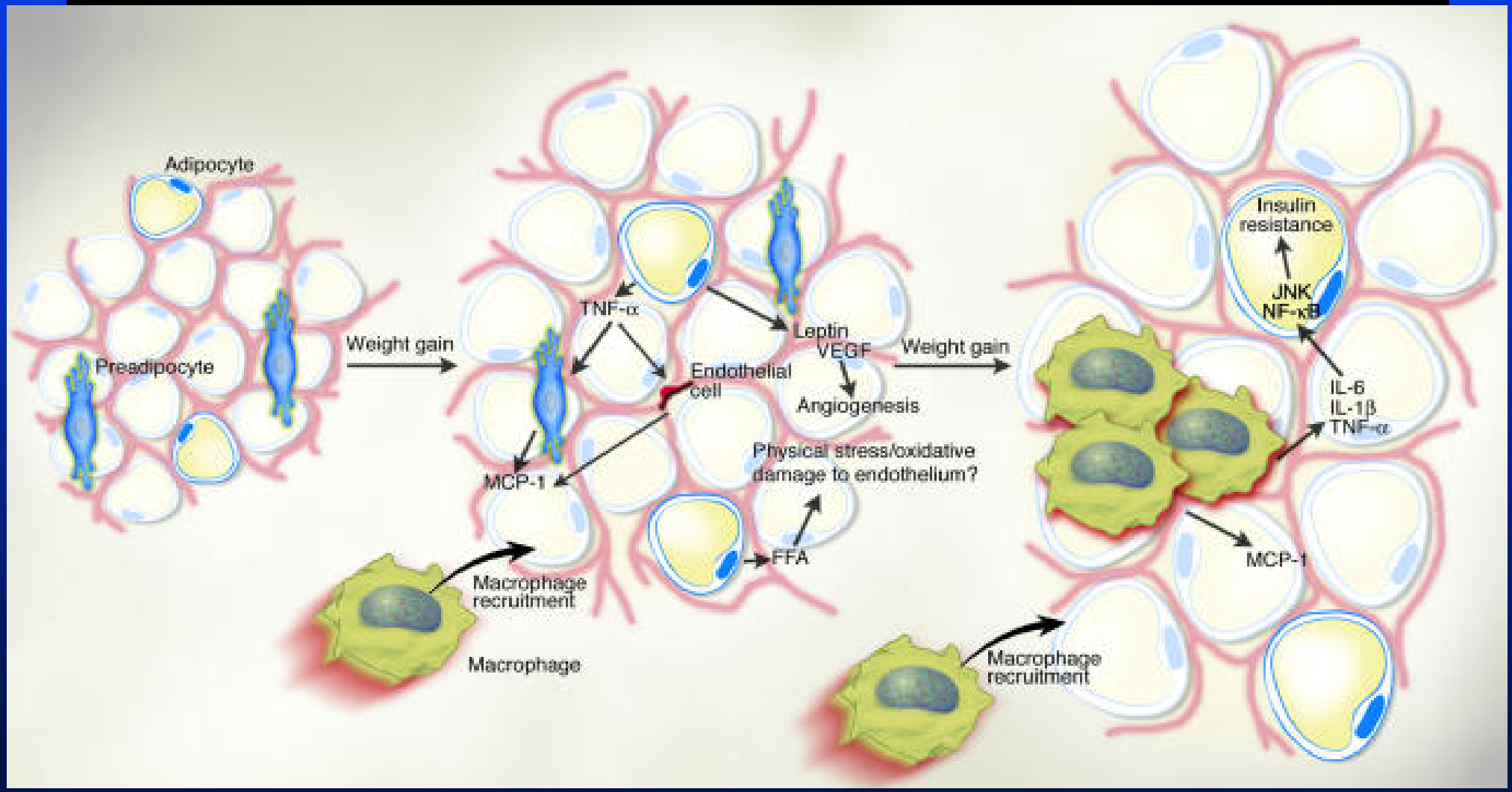
Obesity, Diabetes & Inflammation

Inflamed adipocyte secretome – proteomic signatures

Helen M. Roche,
Nutrigenomics Research Group,
UCD Conway Institute,
University College Dublin,
Ireland

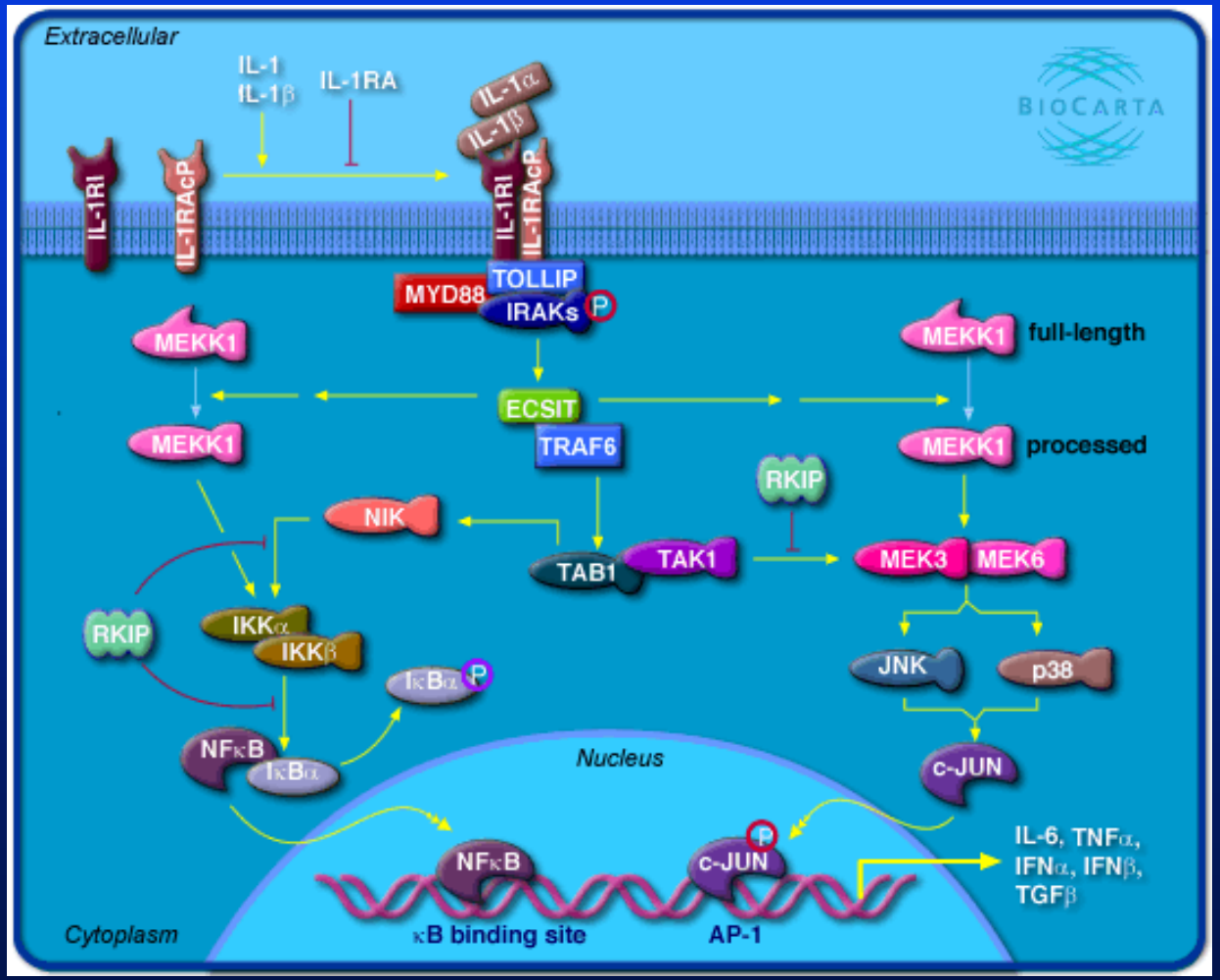


Obesity is a pro-inflammatory state
Macrophage infiltration in adipose tissue



IL-1RI Mediated Inflammation, Obesity & IR

- IL-1β secreted by macrophages & dendritic cells
- IL-1β and IL-1α bind & activate the IL-1 Receptor I (IL-1RI)
- IL-1Ra also binds IL-1RI but has no intrinsic activity
- Activation of IL-1RI results in activation of IKK-NFκB complex & MAPK pathways
- Positive effects with rIL-1Ra on pancreatic β-cell function
- NLRP3 Inflammasome priming & activation of IL-1β by fatty acids



3244 DOI 10.1002/pmic.200800761 *Proteomics* 2009, *9*, 3244–3256

RESEARCH ARTICLE

Attenuation of inflammation and cellular stress-related pathways maintains insulin sensitivity in obese type I interleukin-1 receptor knockout mice on a high-fat diet

Baukje de Ross[1], Vanessa Rungapamestry[2]*, Karen Ross[1], Garry Rucklidge[1], Martin Reid[1], Gary Duncan[1], Graham Horgan[3], Sinead Toomey[2], John Browne[2], Christine E. Loscher[2], Kingston H. G. Mills[4] and Helen M. Roche[2]*

[1] Rowett Institute of Nutrition and Health, University of Aberdeen, Aberdeen, UK
[2] Nutrigenomics Research Group, UCD Conway Institute, School of Public Health & Population Science, University College Dublin, Dublin, Ireland
[3] Biomathematics and Statistics Scotland, Rowett Research Institute, Aberdeen, UK
[4] Immune Regulation Research Group, School of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland

	C57BL6 High fat diet (n=8)	IL-1RI$^{-/-}$ High fat diet (n=8)
Initial Weight (g)	27.575 (0.479)	*24.575 (0.381)
Final Weight (g)	47.75 (0.381)	47.2 (1.81)
EAT weight (g)	0.432 (0.149)	*0.726 (0.299)
Glucose (mmol/L)	13.570 (0.259)	*9.549 (0.614)
Insulin (pmol/L)	349.873 (40.735)	*240.836 (32.139)
HOMA	215.789 (39.715)	*106.074 (14.926)
Quicki	0.284 (0.04)	*0.335 (0.036)
TAG (mmol/L)	0.855 (0.067)	*0.732 (0.064)
Cholesterol (mmol/L)	4.121 (0.517)	3.882 (0.661)
NEFA (mmol/L)	0.696 (0.017)	*0.430 (0.032)
Adiponectin (ng/ml)	7665.6 (609.8)	*9609.5 (833.08)
IL-1 (pg/ml)	27.46 (7.81)	28.56 (5.93)
IL-6 (pg/ml)	1454.9 (82.87)	*737.67 (80.81)
TNFα (pg/ml)	65.65 (7.36)	*16.3 (2.9)

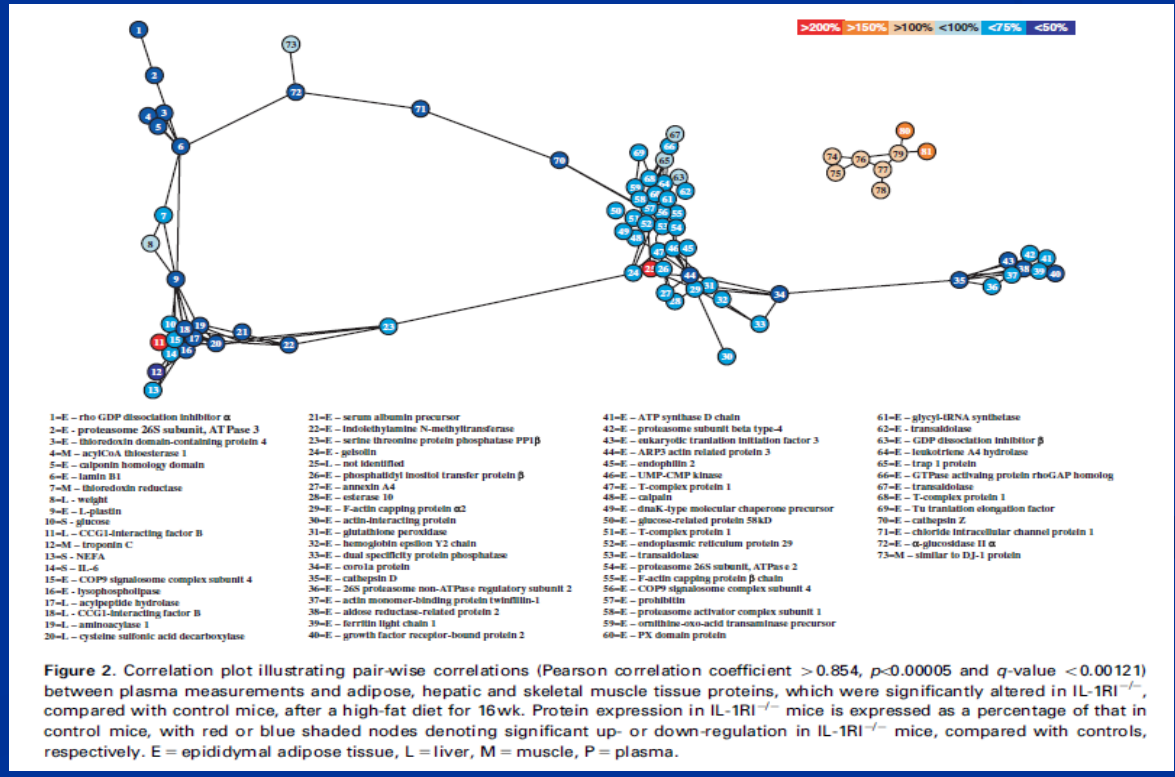


Figure 2. Correlation plot illustrating pair-wise correlations (Pearson correlation coefficient >0.854, p<0.00005 and q-value <0.00121) between plasma measurements and adipose, hepatic and skeletal muscle tissue proteins, which were significantly altered in IL-1RI$^{-/-}$, compared with control mice, after a high-fat diet for 16wk. Protein expression in IL-1RI$^{-/-}$ mice is expressed as a percentage of that in control mice, with red or blue shaded nodes denoting significant up- or down-regulation in IL-1RI$^{-/-}$ mice, compared with controls, respectively. E = epididymal adipose tissue, L = liver, M = muscle, P = plasma.

Lack of Interleukin-1 Receptor I (IL-1RI) Protects Mice From High-Fat Diet–Induced Adipose Tissue Inflammation Coincident With Improved Glucose Homeostasis

Fiona C. McGillicuddy,[1] Karen A. Harford,[1] Clare M. Reynolds,[1] Elizabeth Oliver,[1] Mandy Claessens,[1] Kingston H.G. Mills,[2] and Helen M. Roche[1]

science foundation ireland
fondúireacht eolaíochta éireann

IL-1RI$^{-/-}$ Time Course *In Vivo* Study Design

Commencement of HFD or LFD diet in WT and IL-1RI$^{-/-}$ animals

Baseline - Week 0 (6wks old)
GTT and ITT,
adipose tissue culture;
SVF isolation and flow cytometry sac animals and harvest tissues

Week 6
GTT/ITT on both chow and HFD animals;
SVF isolation and flow cytometry
Sac animals and harvest tissues

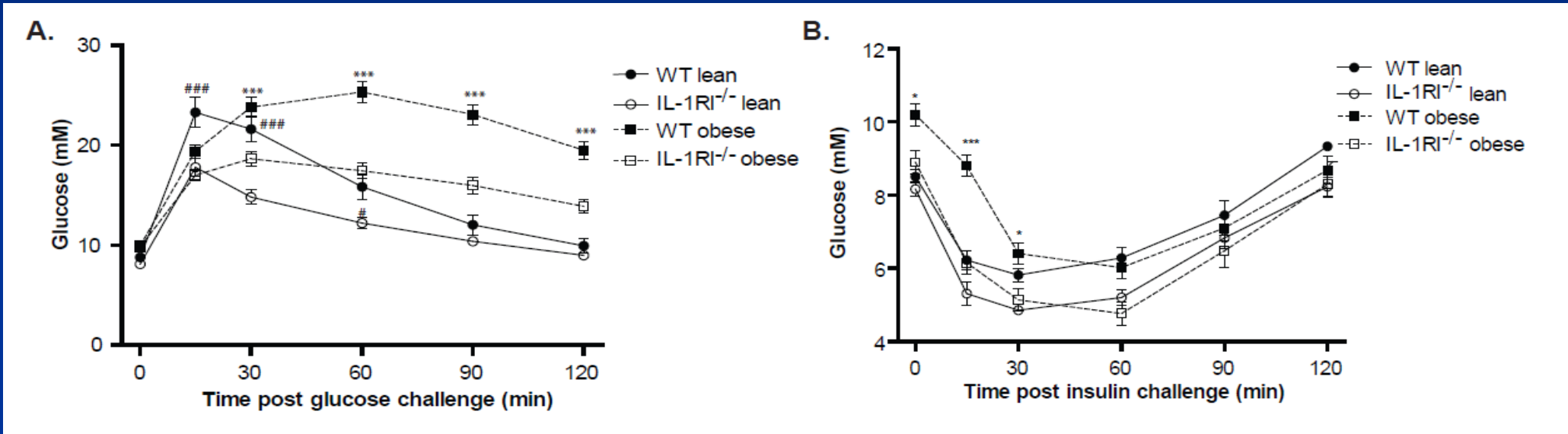
Week 12
GTT/ITT on HFD animals;
SVF isolation and flow cytometry
Sac animals and harvest tissues

Week 16
GTT/ITT on both chow and HFD animals,
adipose tissue culture;
SVF isolation and flow cytometry
Sac animals

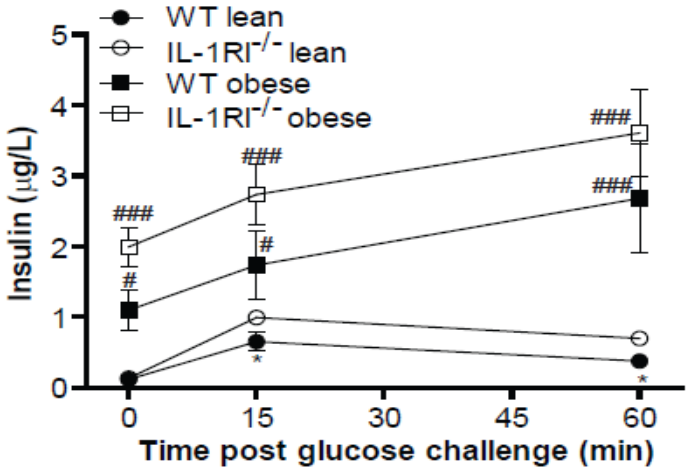
GTT: Tail-tips amputated and baseline glucose levels measured using a glucometer Mice injected with 1.5g/kg (25% w/v) glucose and clearance from plasma monitored over 120min
ITT: Mice injected with 0.75U/kg insulin and glucose levels were monitored over 120min

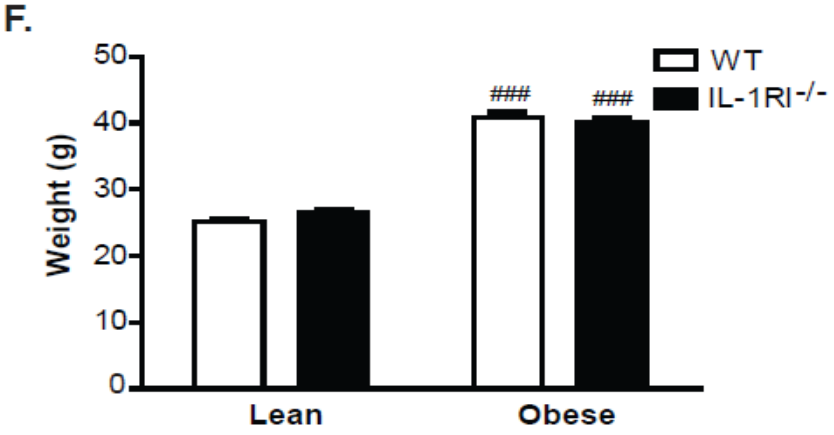
	High-fat diet			Low-fat diet	
Product #	**D07081501**			**D07081502**	
	gm (%)	*kcal (%)*		gm (%)	*kcal (%)*
Protein	24	*20*		19	*20*
Carbohydrate	41	*35*		67	*70*
Fat	24	*45*		4	*10*
Total		*100*			*100*
kcal/gm	4.73			3.85	
Ingredient	**gm**	***kcal***		**gm**	***kcal***
Casein, 80 Mesh	200	*800*		200	*800*
L-Cystine	3	*12*		3	*12*
Corn Starch	72.8	*291*		315.0	*1260*
Maltodextrin 10	100.0	*400*		35.0	*140*
Sucrose	172.8	*691*		350.0	*1400*
Cellulose, BW200	50	*0*		50	*0*
Soybean Oil	25.0	*225*		25	*225*
Lard	0.0	*0*		0	*0*
Palm Oil	177.5	*1598*		20	*180*
Mineral Mix S10026	10	*0*		10	*0*
DiCalcium Phosphate	13	*0*		13	*0*
Calcium Carbonate	5.5	*0*		5.5	*0*
Potassium Citrate, 1 H	16.5	*0*		16.5	*0*
Vitamin Mix V10001	10	*40*		10	*40*
Choline Bitartrate	2	*0*		2	*0*
FD&C Yellow Dye #5	0.025	*0*		0.025	*0*
FD&C Red Dye #40	0	*0*		0.025	*0*
FD&C Blue Dye #1	0.025	*0*		0	*0*
Total	**858.15**	***4057***		**1055.05**	***4057***

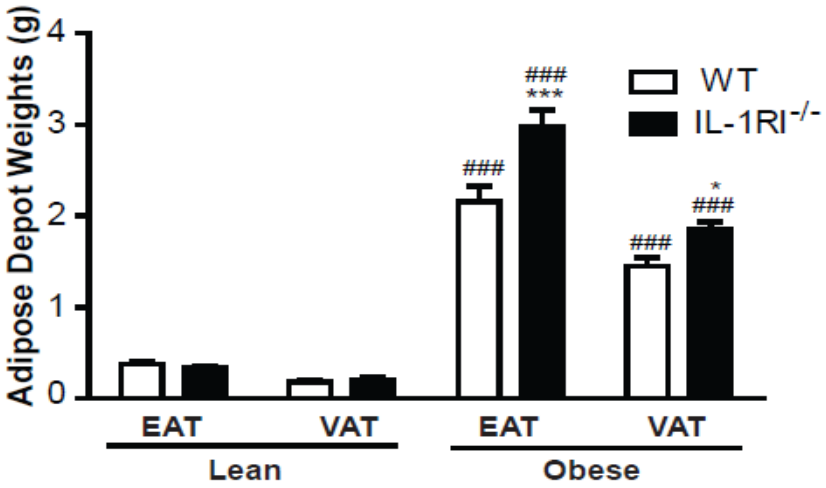
IL-1RI$^{-/-}$ mice are protected against high-fat diet induced insulin resistance

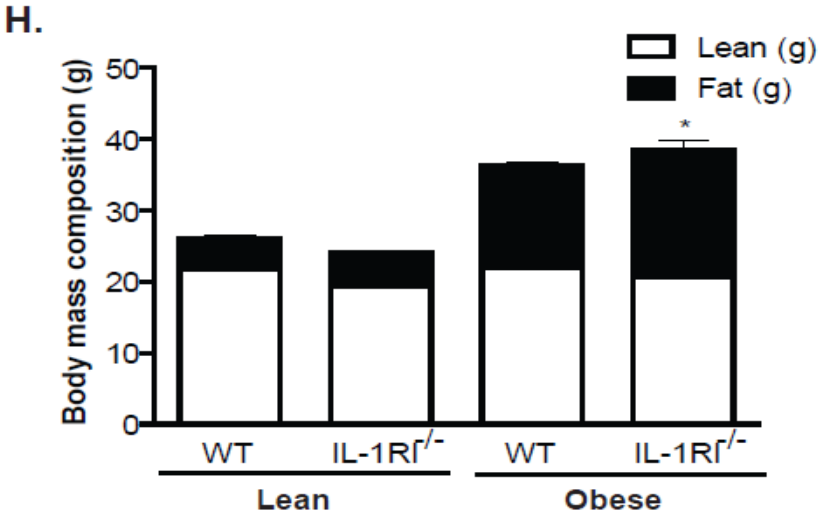


Despite improved glucose homeostasis IL-1RI$^{-/-}$ mice are hyperinsulinemic and have increased adipose mass

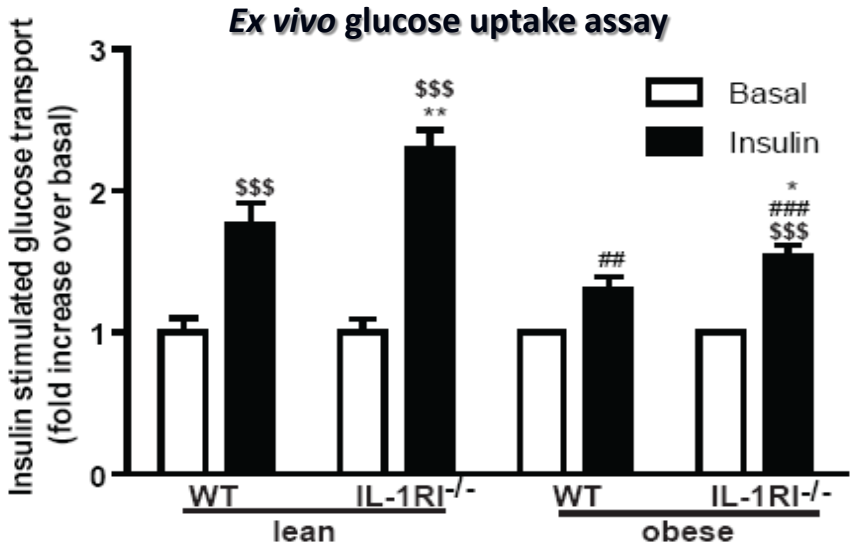


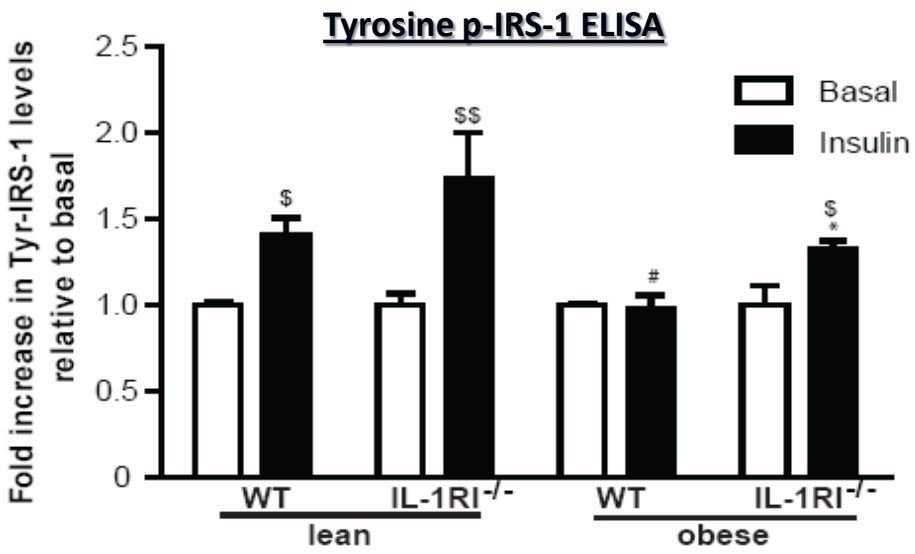


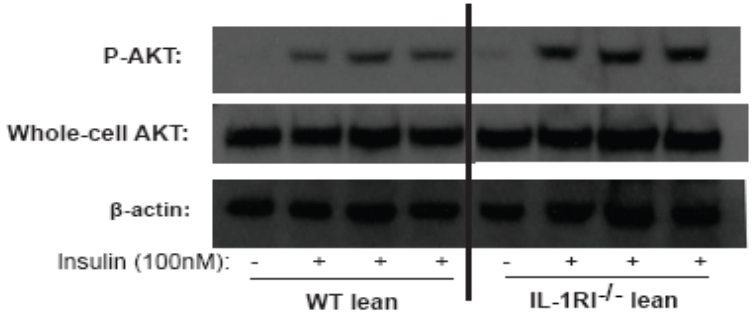


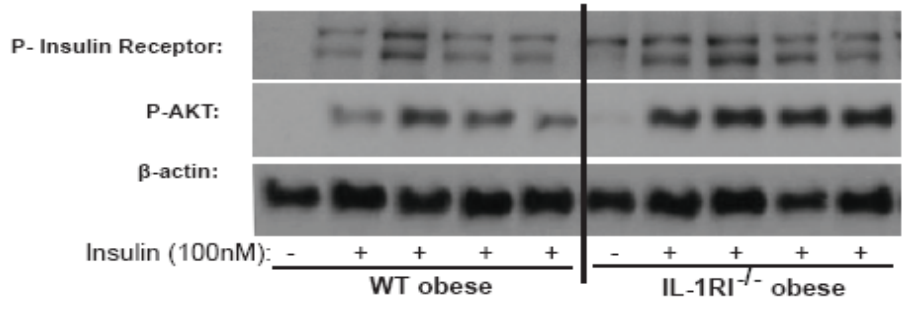


IL-1RI$^{-/-}$ adipose is more insulin sensitive than WT both at baseline and after HFD

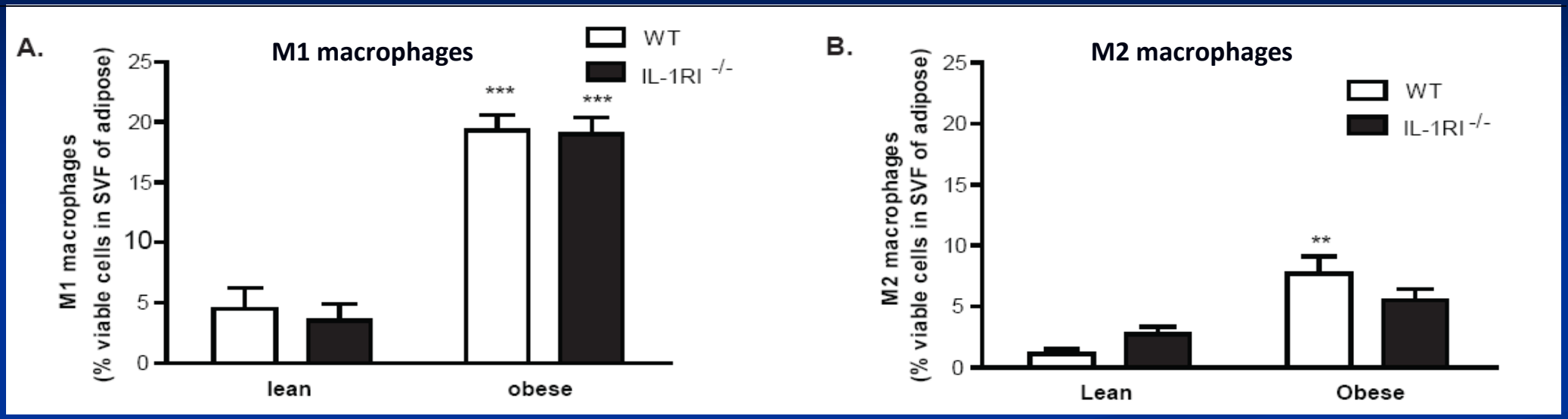


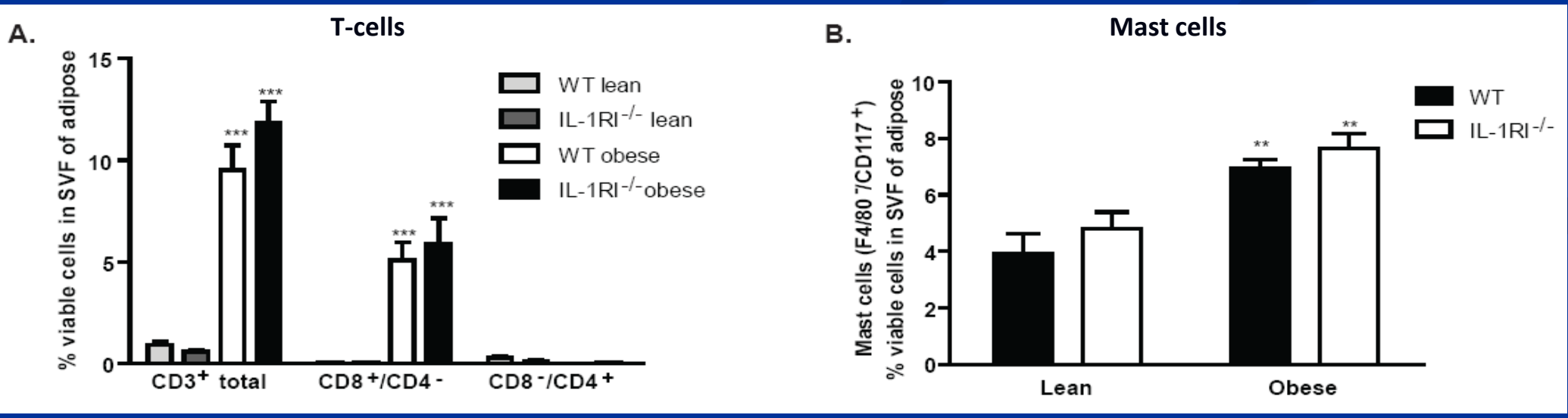




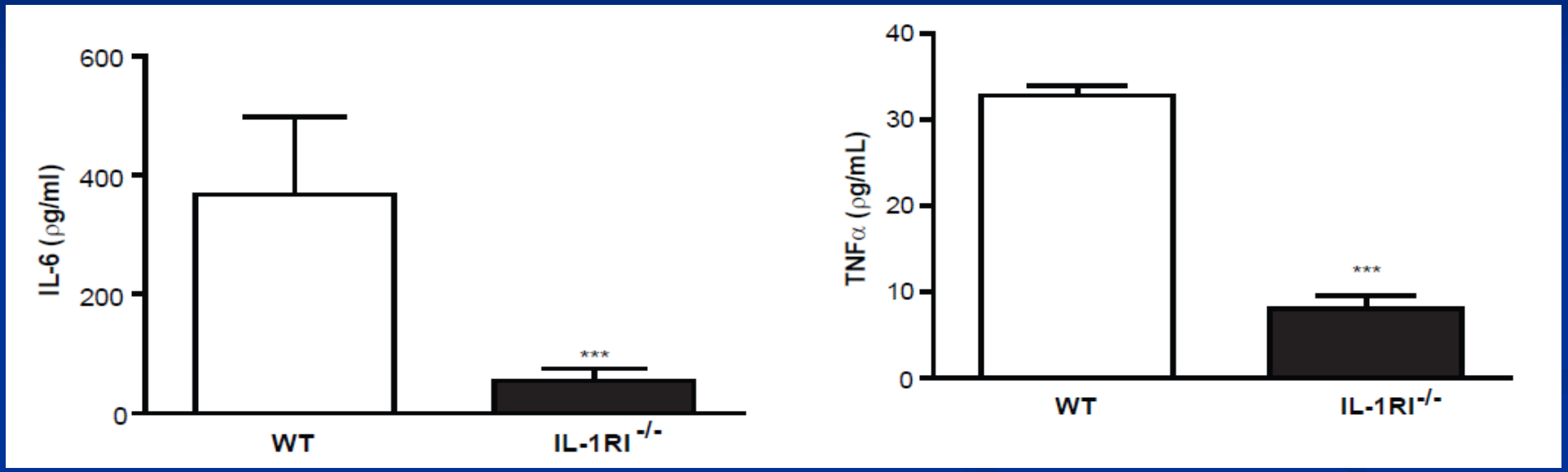


Equivalent immune cell recruitment into adipose

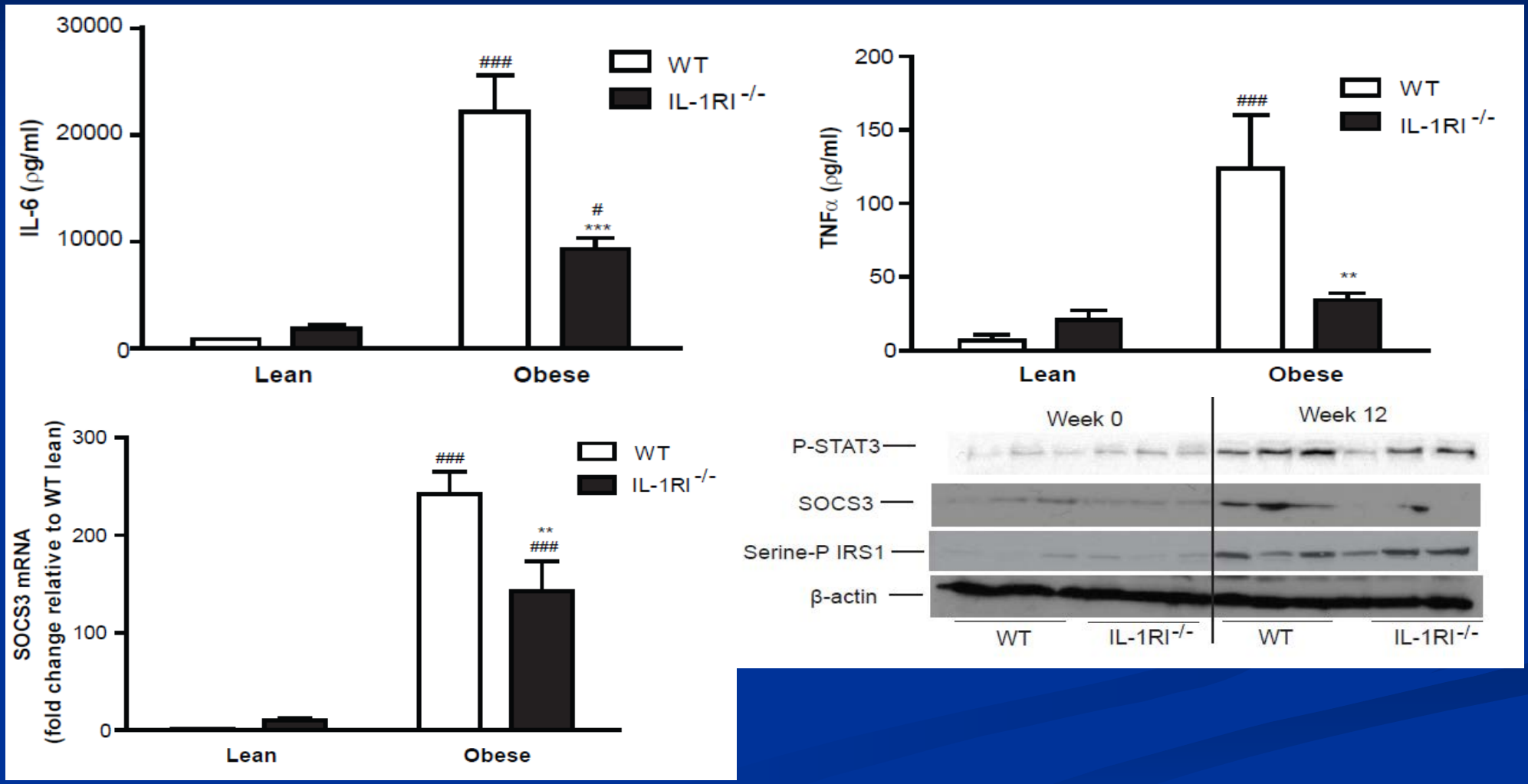




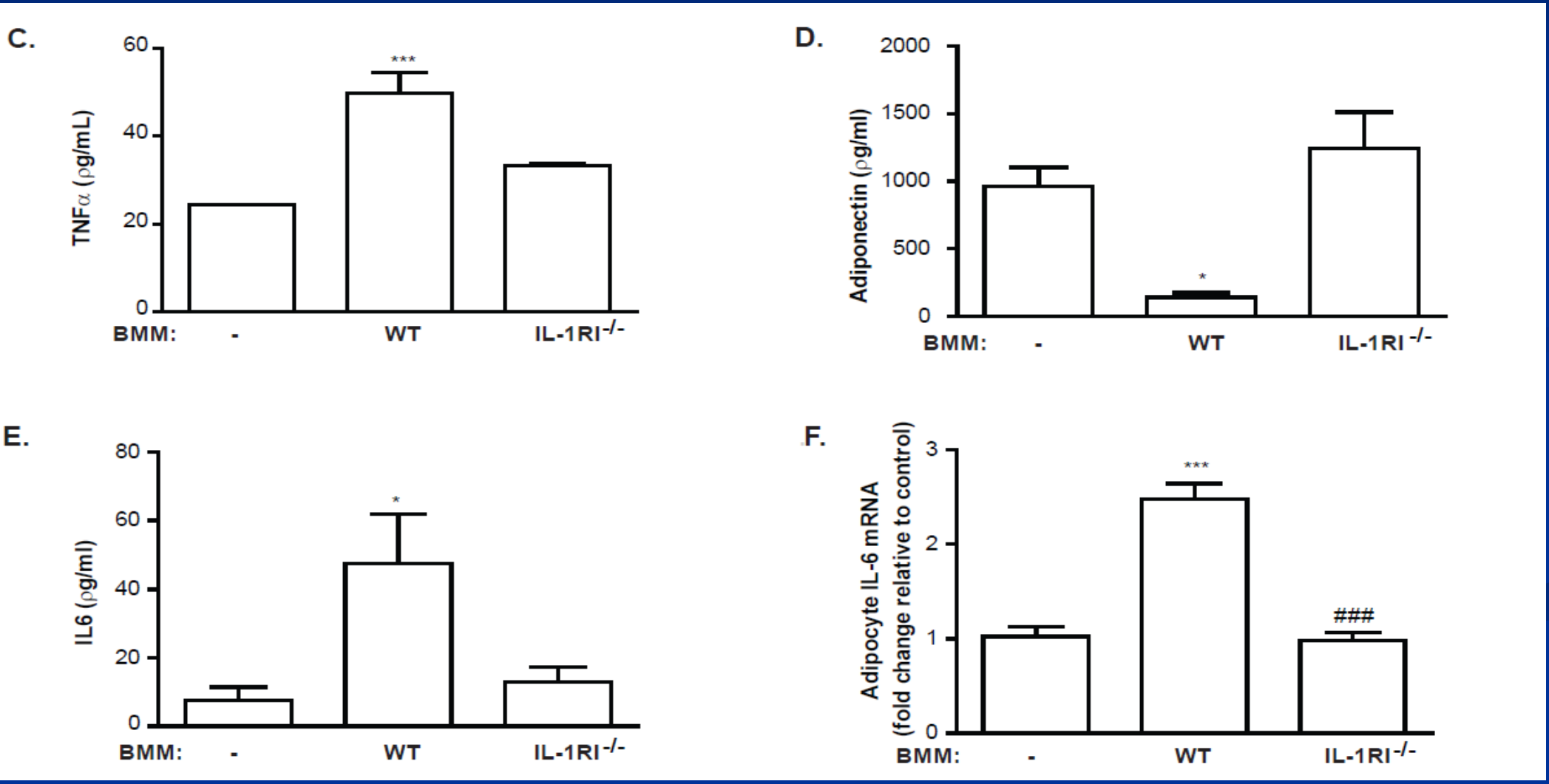
IL-1RI$^{-/-}$ adipose tissue macrophages are less immunogenic than WT



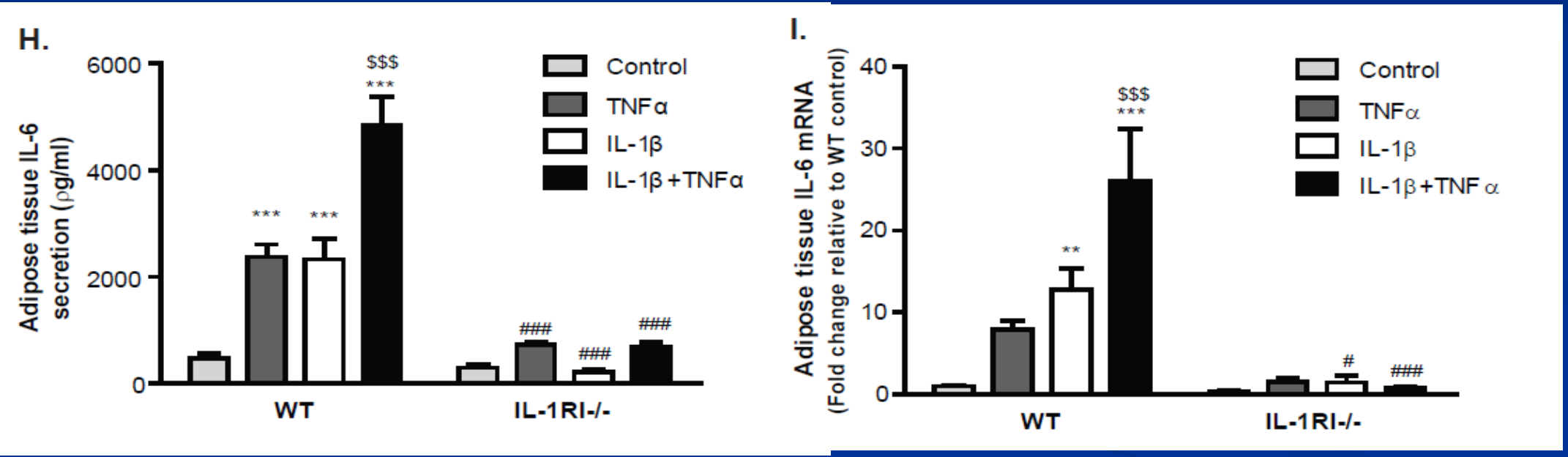
Attenuated inflammation in IL-1RI$^{-/-}$ adipose explants after high-fat diet



Altered cytokine cross-talk between IL-1RI$^{-/-}$ macrophages and adipocytes



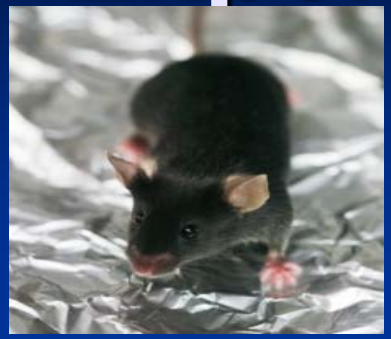
IL-1β and TNFα synergy in adipose tissue is lost in absence of IL-1RI



Exploratory analysis of the Adipose Tissue Secretome

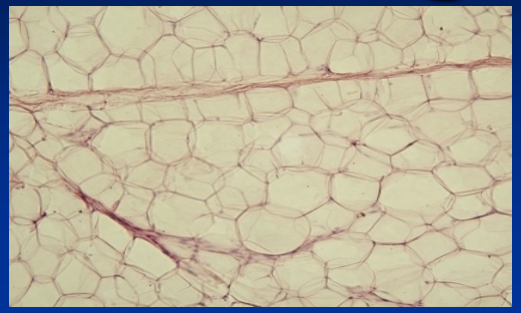
Eugene Dillon
Dr Fiona McGilicuddy
Prof Giuliano Elia
Prof Helen Roche

Experimental Design & Workflow



WT & IL1RKO

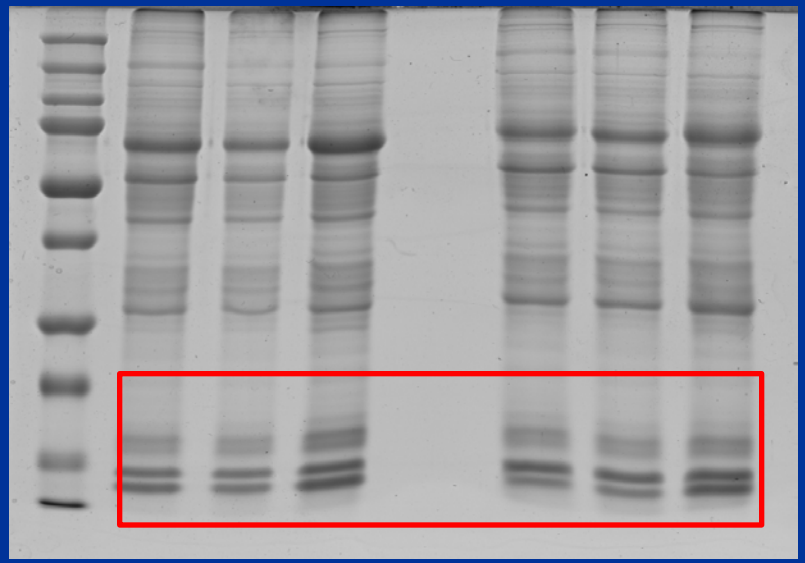
12wk HF Diet



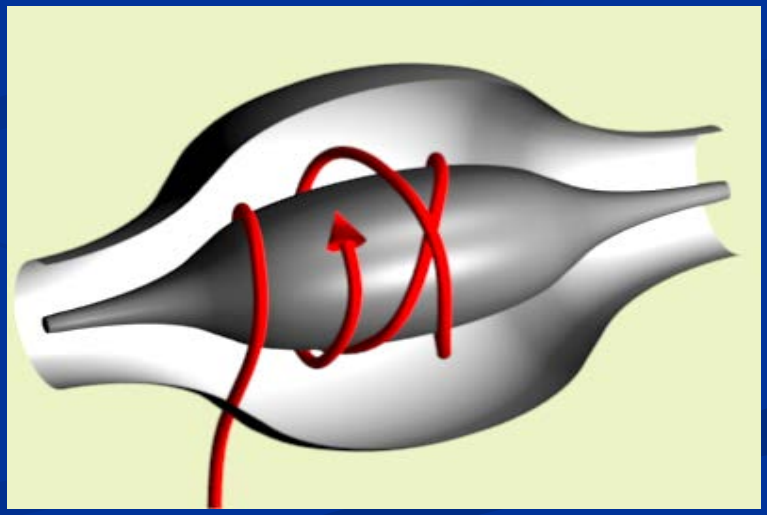
Adipose Tissue Explant, Cultured 6hr

Protein isolated & run on SDS PAGE

WT1 WT2 WT3 KO1 KO2 KO3

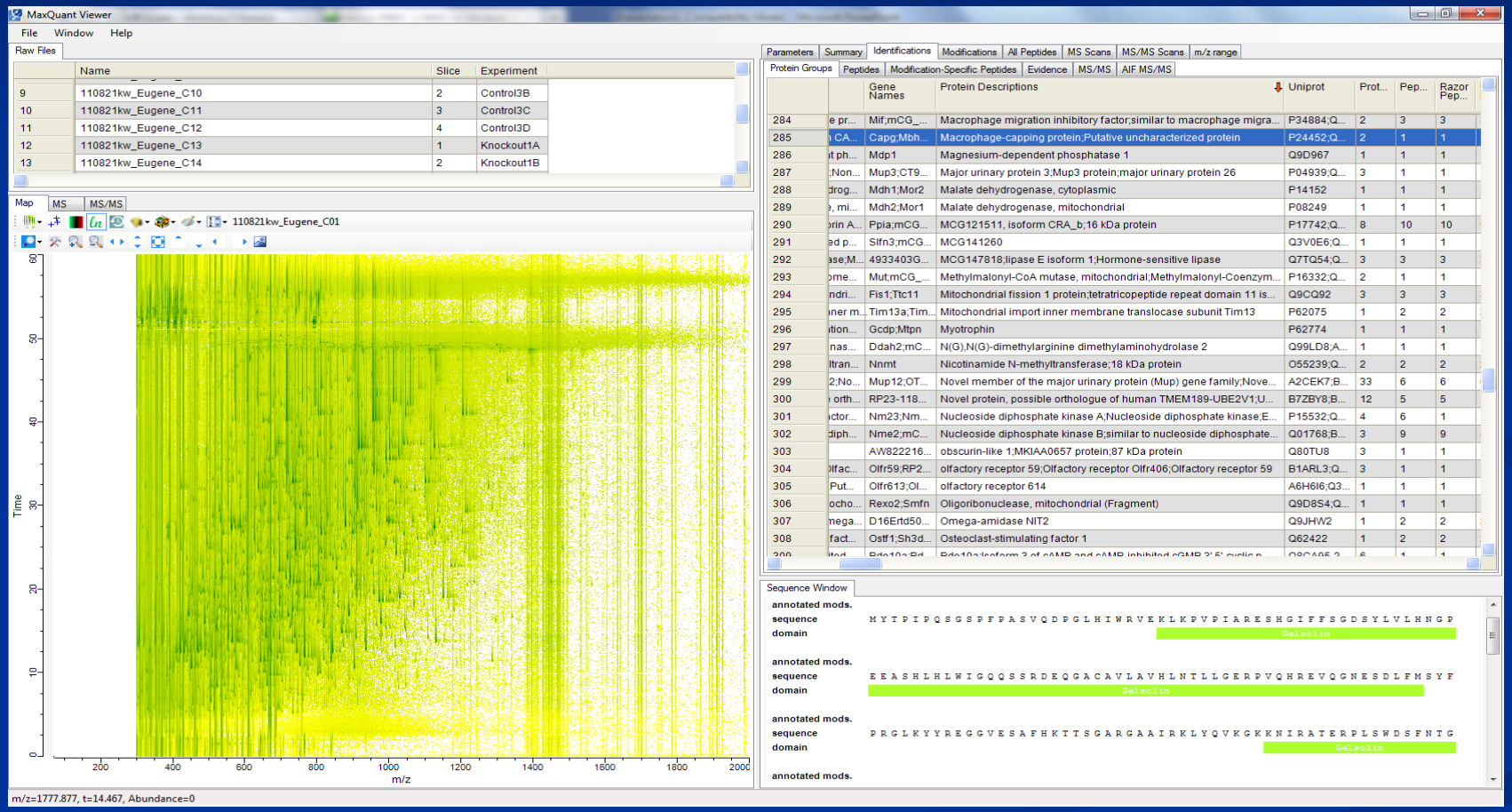


Slices representing 5kDa-25kDa Tryptic digestion



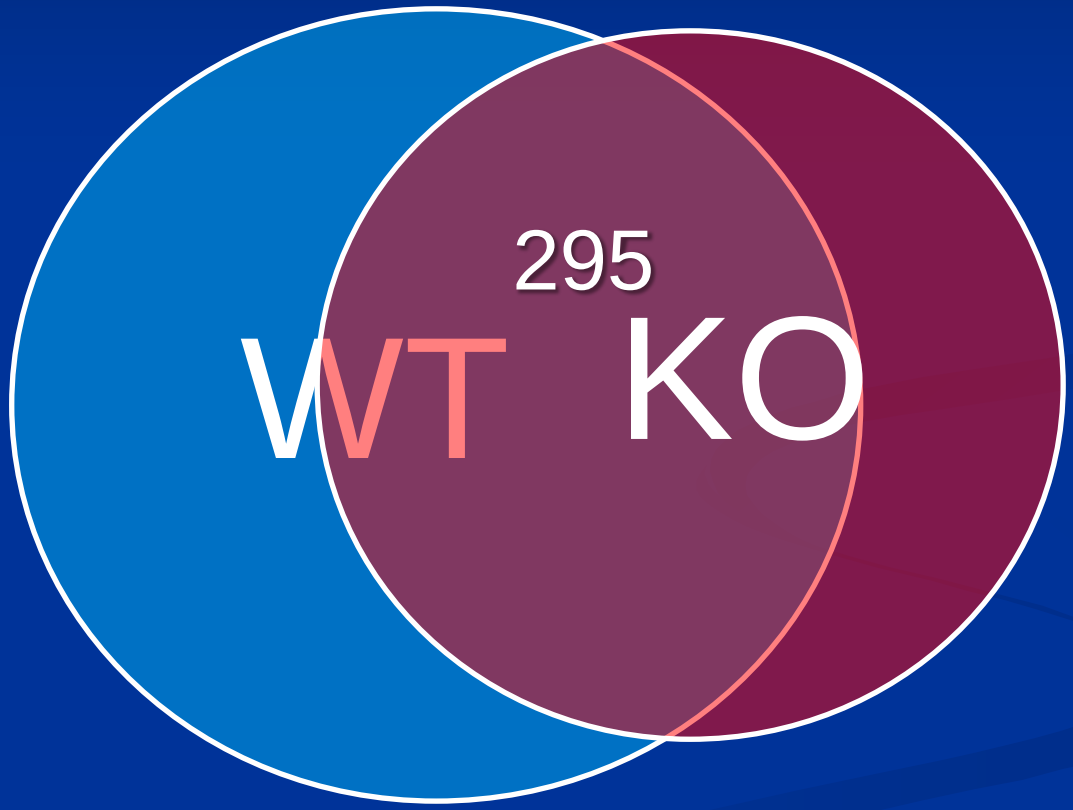
LC-MS/MS Orbitrap

Adipose Tissue Secretome with MaxQuant



MaxQuant FDR 0.05% identified 405 proteins

Huge overlap in proteins expressed



295 proteins overlapped with WT and KO only expressing 70 and 41 different proteins

Adipose secretome - proteins reduced in IL-1RI$^{-/-}$ compared to WT

Majority Protein IDs	Protein Names	Uniprot	RAZ PEP FOLD CHANGE	SIGNAL PEP
IPI00132575	Coactosin-like protein	Q9CQI6	7.0	
IPI00111013	Cathepsin D	P18242	6.0	Y
IPI00320239	C-type lectin domain family 3 member B	P43025	5.5	Y
IPI00944681	Major urinary protein 12	A2CEK7	5.0	Y
IPI00378649	Major urinary protein 24	Q5FW60	5.0	Y
IPI00853914	Heterogeneous nuclear ribonucleoproteins A2/B1	O88569-1	5.0	
IPI00123924	Alpha-1 protease inhibitor 4	Q00897	5.0	Y
IPI00139788	Beta-1 metal-binding globulin	Q921I1	4.0	Y
IPI00173343	Oligoribonuclease, mitochondrial	Q9D8S4	4.0	
IPI00649972	Vacuolar protein sorting 25 (Yeast)	A2A4J8	4.0	
IPI00133948	13 kDa FK506-binding protein	P45878	4.0	Y
IPI00756745	Lama2 protein	Q7TQI9	4.0	
IPI00229680	Calcineurin homologous protein	P61022	4.0	
IPI00130640	Heat-responsive protein 12	P52760	4.0	
IPI00319830	Beta-II spectrin	Q62261-1	3.7	
IPI00114368	ER-Golgi SNARE of 24 kDa	O08547	3.5	
IPI00134621	GTPase Ran	P62827	3.3	
IPI00331442	Peptide methionine sulfoxide reductase	Q9D6Y7	3.3	
IPI00230185	Glycerol-3-phosphate dehydrogenase [NAD+], cytoplasmic	P13707	3.0	
IPI00655110	Epididymal retinoic acid-binding protein	A2AJB7-1	3.0	
IPI00125931	Cystatin-B	Q62426	3.0	

Adipose secretome - proteins reduced in IL-1RI$^{-/-}$ compared to WT

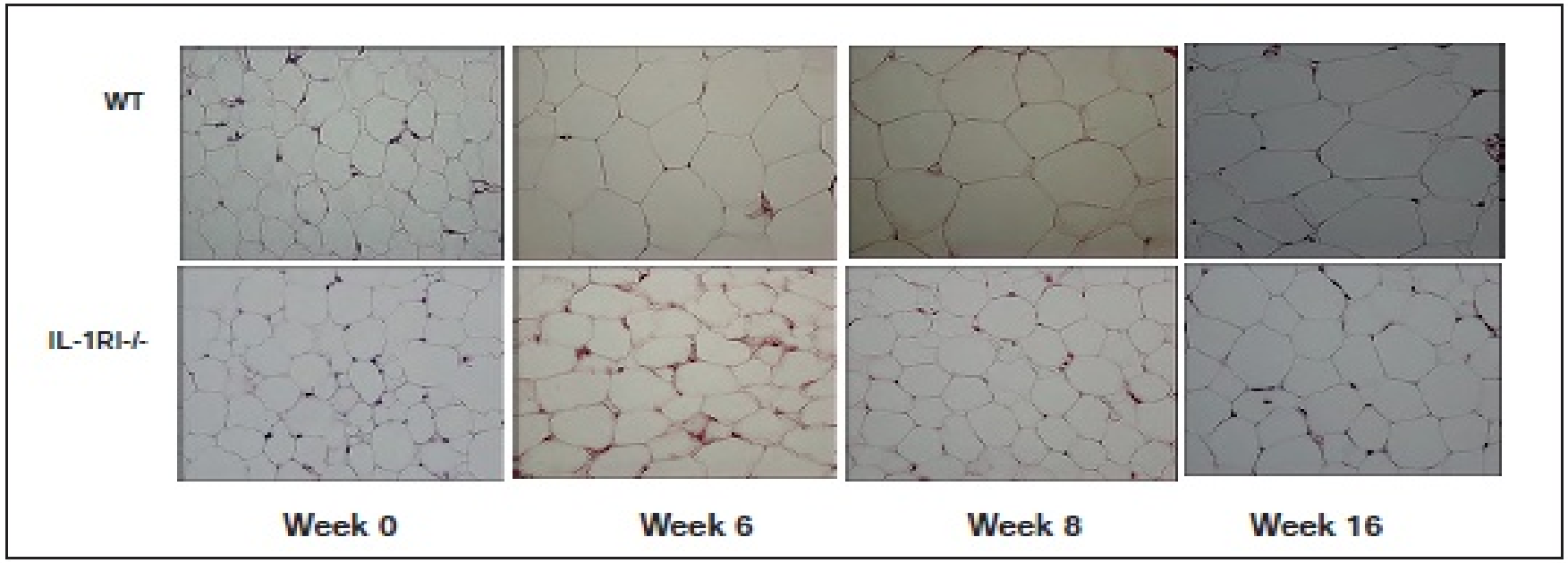
- Cathepsin D, S & C expression reduced in IL-1RI $^{-/-}$
- Cathepsins are cysteine proteases, regulated by Nf-kB and initiate inflammatory cascades
- Cath-B activates caspase 11, cleaves caspase 1 mediating release of mature IL-1β.

- Elevated in obesity and IR & may play a role in adipogenesis
- Cath-D expression is up-regulated in obesity & during adipocyte differentiation
- Cath-D silencing impedes adipogenesis and inhibits PPARγ, HSL and aP2 expression.

- Cathepsins also function to modify the extracellular matrix
- Adipose morphology – marked difference

Adipocyte hypertrophy evident in WT mice with HFD but not evident in IL-1RI$^{-/-}$ mice

A. Adipocyte size in WT and IL-1RI-/- adipose tissue during high fat feeding



Adipose secretome - proteins increased in IL-1RI$^{-/-}$ compared to WT

Majority Protein IDs	Protein Names	Uniprot	RAZ PEP FOLD CHANGE
IPI00229475	Desmoplakin III	Q02257	10
IPI00930843	Bromodomain adjacent to zinc finger domain protein 1Bsyndrome chromosomal region 9 protein homolog	Q9Z277-2	6
IPI00124223	11S regulator complex subunit alpha	P97371	5
IPI00120465	Resistin	Q99P87	5
IPI00118286	14-3-3 protein sigma	O70456	4.75
IPI00118384	14-3-3 protein epsilon	P62259	4.666666667
IPI00459279	Dihydropteridine reductase	Q8BVI4	4
IPI00928375	Hormone-sensitive lipase	Q7TQ54	4
IPI00117829	Caveolin-1	P49817-1	3.666666667
IPI00555071	Ras suppressor protein 1	Q01730	3.571428571
IPI00230707	14-3-3 protein gamma	P61982	3.533333333
IPI00223783	Lipid droplet-associated protein	Q8CGN5-1	3.2
IPI00116498	14-3-3 protein zeta/delta	P63101	3.0625
IPI00853924	14-3-3 protein tau	P68254-1	3
IPI00759878	Complement C3	P01027-2	3
IPI00230320	Carbonate dehydratase I;Carbonic anhydrase 1	P13634	3
IPI00225390	Cytochrome c oxidase subunit 6B1	P56391	3
IPI00114329	Gamma-ECS regulatory subunit	O09172	3
IPI00115866	Glyoxalase II	Q99KB8-1	3
IPI00123449	Spot 14 protein	Q62264	3

Adipose secretome - proteins increased in IL-1RI$^{-/-}$ compared to WT

- 14-3-3 protein isoforms
  - Apoptosis, ER Stress, MMP-1, cellular re-modelling
  - 14-3-3 signal transduction-regulatory protein
  - Many AKT substrates undergo 14-3-3 binding upon phosphorylation, this may modulate AKT action
  - Regulate plasma membrane GLUT4 content and insulin-stimulated glucose uptake.

Functional effect of adipose secretome

- Clear synergy between IL-1β & TNFα with functional effects on adipocyte IR
- Coupled with important ECM re-modelling
- In terms of the regulatory mechanisms that determine adipose morphology
  - ECM re-modelling important
    - a function or consequence of lack of IL-1RI
  - Lack of IL-1RI affects adipogenic potential

Acknowledgements

- **Nutrigenomics Research Group**
  - Fiona McGillicuddy
  - Eugene Dillon
  - Clare Reynolds
  - Karen Hartford
  - Orla Finucane
  - Colm O'Grada
  - Melissa Morine
  - Catherine Phillips
  - Jane Ferguson
  - Audrey Tierney
  - Jolene Mc Monagle
  - Vanessa Rungapamestry
  - Annalouise O'Connor
  - Elizabeth Oliver
- **Trinity College Dublin**
  - James Gibney
  - Kingston Mills
- **Rowett Research Institute**
  - Baukje De Roos
- **University College Dublin**
  - Alfonso Blanco
  - Gerard Cagney & Giuliano Elia
  - Lorraine Brennan & Mike Gibney

- **Science Foundation Ireland**
  - **Principal Investigator Programme**
- **Dept of Agriculture & Food**
  - **Food Institutional Research Measure**
- **EU Framework 6**
  - **Food Quality & Safety Programme**
- **Health Research Board**
  - **PhD Mol Med Programme**