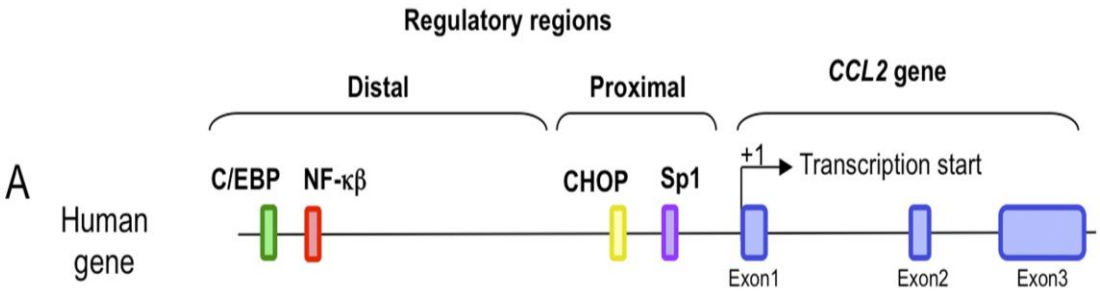
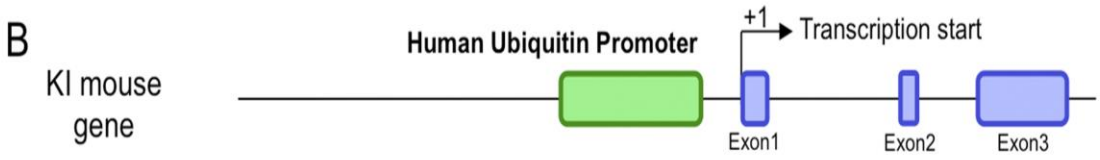
Supplementary material S1, comparison between human and murine CCL2 regions, corresponding to the manuscript:

Ubiquitous transgenic overexpression of C-C chemokine ligand 2: a model to assess the combined effect of high energy intake and continuous low-grade inflammation?

Esther Rodríguez-Gallego, Marta Riera-Borrull, Anna Hernández-Aguilera, Roger Mariné-Casadó, Anna Rull, Raúl Beltrán-Debón, Fedra Luciano-Mateo, Javier A. Menéndez[1], Alejandro Vazquez-Martin[1], Juan J. Sirvent[2], Vicente Martín-Paredero[3], Angel L. Corbí[4], Elena Sierra-Filardi[4], Gerard Aragonès, Anabel García-Heredia, Jordi Camps, Carlos Alonso-Villaverde[5], Jorge Joven*

A, displays a schematic view of human *CCL2* gene region that includes its regulatory region and the most prominent elements identified. The *Ccl2* gene transgenic vector includes the murine CCL2 gene and the comparative position of human ubiquitin promoter (B); sequence is provided in C.





C

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3781 TGGGGCGGTG AACGCCGATG ATTATATAAG GACGCGCCGG GTGTGGCACA GCTAGTTCCG
3841 TCGCAGCCGG GATTTGGGTC GCGGTTCTTG TTTGTGGATC GCTGTGATCG TCACTTGGTG
3901 AGTAGCGGGC TGCTGGGCTG GCCGGGGCTT TCGTGGCCGC CGGGCCGCTC GGTGGGACGG
3961 AAGCGTGTGG AGAGACCGCC AAGGGCTGTA GTCTGGGTCC GCGAGCAAGG TTGCCCTGAA
4021 CTGGGGGTTG GGGGGAGCGC AGCAAAATGG CGGCTGTTCC CGAGTCTTGA ATGGAAGACG
4081 CTTGTGAGGC GGGCTGTGAG GTCGTTGAAA CAAGGTGGGG GGCATGGTGG GCGGCAAGAA
4141 CCCAAGGTCT TGAGGCCTTC GCTAATGCGG GAAAGCTCTT ATTCGGGTGA GATGGGCTGG
4201 GGCACCATCT GGGGACCCTG ACGTGAAGTT TGTCACTGAC TGGAGAACTC GGTTTGTCGT
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4321 CGCGCGCCCT CGTCGTGTCG TGACGTCACC CGTTCTGTTG GCTTATAATG CAGGGTGGGG
4381 CCACCTGCCG GTAGGTGTGC GGTAGGCTTT TCTCCGTCGC AGGACGCAGG GTTCGGGCCT
4441 AGGGTAGGCT CTCCTGAATC GACAGGCGCC GGACCTCTGG TGAGGGGAGG GATAAGTGAG
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4561 TTGAACTATG CGCTCGGGGT TGGCGAGTGT GTTTTGTGAA GTTTTTTAGG CACCTTTTGA
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4681 ATTCTGGCCG TTTTTGGCTT TTTTGTTAGA CGCCACCATG CATATAACTT CGTATAGCAT
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Supplementary material S2, details of the sequence of other main elements used in the construction of the vector, corresponding to the manuscript:

Ubiquitous transgenic overexpression of C-C chemokine ligand 2: a model to assess the combined effect of high energy intake and continuous low-grade inflammation?

Esther Rodríguez-Gallego, Marta Riera-Borrull, Anna Hernández-Aguilera, Roger Mariné-Casadó, Anna Rull, Raúl Beltrán-Debón, Fedra Luciano-Mateo, Javier A. Menéndez[1], Alejandro Vazquez-Martin[1], Juan J. Sirvent[2], Vicente Martín-Paredero[3], Angel L. Corbí[4], Elena Sierra-Filardi[4], Gerard Aragonès, Anabel García-Heredia, Jordi Camps, Carlos Alonso-Villaverde[5], Jorge Joven*

The sequence of main elements used in the construction of the vector is described according to the following, color-based legend. For clarity, flanking regions were also included.

5'homology arm 465-3467
MCS 3475-3495
UbiC promoter 3500-4709
loxP site 4724-4757
STOP cassette 4768-6101
loxP site 6102-6135
genomic fragment of *CCL2* gene (frag) 6150-9056
exon1 6468-6628
exon2 7374-7491
exon3 7817-8343
SV40pA 9090-9318
FRT site 9331-9378
PGK promoter 9388-9906
Neo CDS 9974-10777
Splice donor (SD) 10799-11005
Instability signal (IS) 11006-11059
FRT site 11090-11137
MCS 11209-11230
3'homology arm 11231-14260

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   121 GACGGGGAAA GCCGGCGAAC GTGGCGAGAA AGGAAGGGAA GAAAGCGAAA GGAGCGGGCG
   181 CTAGGGCGCT GGCAAGTGTA GCGGTCACGC TGCGCGTAAC CACCACACCC GCCGCGCTTA
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   301 GATCGGTGCG GGCCTCTTCG CTATTACGCC AGCTGGCGAA AGGGGGATGT GCTGCAAGGC
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   421 AATTGTAATA CGACTCACTA TAGGGCGAAT TGGGTACCGG CGCGCCTAGC GACCTAAACC
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   541 CCTCCTGGAA CCTCCTCCAT CTGGATCCTC CCCAATCAAA AGTATAGGTA TTTAACATAT
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841 ACGCACCCCT GACCCAGGCC AGCAGGGCGG GCTGCAGCAT CAGTACACAG GAGAAAGATC

901 CTTATTCCTA AGAATGAGAA AGGCAAAGGC GCCCGATAGA ATAAATTAGC ATAGAAGGGG

961 CTTTCCCAGG AGTTAAAACT TTCCTTCTGA GCGATTACCT ACTAAAACCA GGGCTTTTGC

1021 CCACTACCAT TTACCTAGGA TCTTGGCTTG CACGGATTCA TAGGGGCATA TCCCTCCCCC

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1141 AATCTGGCTC AATTCCAGGC TAGAACCCTA CAAATTCAAC AGGGATATCG CAAGGATACT

1201 GGGGCATACG CCACAGGGAG TCCAAGAATG TGAGGTGGGG GTGGCGAAGG TAATGTCTTT

1261 GGTGTGGGAA AAGCAGCAGC CATCTGAGAT AGGAACTGGA AAACCAGAGG AGAGGCGTTC

1321 AGGAAGATTA TGGAGGGGAG GACTGGGCCC CCACGAGCGA CCAGAGTTGT CACAAGGCCG

1381 CAAGAACAGG GGAGGTGGGG GGCTCAGGGA CAGAAAAAAA AGTATGTGTA TTTTGAGAGC

1441 AGGGTTGGGA GGCCTCTCCT GAAAAGGGTA TAAACGTGGA GTAGGCAATA CCCAGGCAAA

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1981 GCGGAGGGGG CCGGCACTAC TGTGTTGGCG GACTGGCGGG ACTAGGGCTG CGTGAGTCTC

2041 TGAGCGCAGC GGGCGGCGGC CGCCCCTCCC CCGGCGGCGG CAGCGGCGGC AGCGGCGGCA

2101 GCTCACTCAG CCCGCTGCCC GAGCGGAAAC GCCACTGACC GCACGGGGAT TCCCAGTGCC

2161 GGCGCCAGGG GCACGCGGGA CACGCCCCCT CCCGCCGCGC CATTGGCCTC TCCGCCCACC

2221 GCCCCACACT TATTGGCCGG TGCGCCGCCA ATCAGCGGAG GCTGCCGGGG CCGCCTAAAG

2281 AAGAGGCTGT GCTTTGGGGC TCCGGCTCCT CAGAGAGCCT CGGCTAGGTA GGGGATCGGG

2341 ACTCTGGCGG GAGGGCGGCT TGGTGCGTTT GCGGGGATGG GCGGCCGCGG CAGGCCCTCC

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3241 GGAGGCAGGA AGCACTTGCT CTCCCAAAGT CGCTCTGAGT TGTTATCAGT AAGGGAGCTG

3301 CAGTGGAGTA GGCGGGGAGA AGGCCGCACC CTTCTCCGGA GGGGGGAGGG GAGTGTTGCA

3361 ATACCTTTCT GGGAGTTCTC TGCTGCCTCC TGGCTTCTGA GGACCGCCCT GGGCCTGGGA

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11461 TCGGAGTATT TTCCATCGAG GTAGATTAAA GACATGCTCA CCCGAGTTTT ATACTCTCCT

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11581 ATTTTAATCA TTTTTAAAGA GCCCAGTACT TCATATCCAT TTCTCCCGCT CCTTCTGCAG

11641 CCTTATCAAA AGGTATTTTA GAACACTCAT TTTAGCCCCA TTTTCATTTA TTATACTGGC

11701 TTATCCAACC CCTAGACAGA GCATTGGCAT TTTCCCTTTC CTGATCTTAG AAGTCTGATG

11761 ACTCATGAAA CCAGACAGAT TAGTTACATA CACCACAAAT CGAGGCTGTA GCTGGGGCCT

11821 CAACACTGCA GTTCTTTTAT AACTCCTTAG TACACTTTTT GTTGATCCTT TGCCTTGATC

11881 CTTAATTTTC AGTGTCTATC ACCTCTCCCG TCAGGTGGTG TTCCACATTT GGGCCTATTC

11941 TCAGTCCAGG GAGTTTTACA ACAATAGATG TATTGAGAAT CCAACCTAAA GCTTAACTTT

12001 CCACTCCCAT GAATGCCTCT CTCCTTTTTC TCCATTTATA AACTGAGCTA TTAACCATTA

12061 ATGGTTTCCA GGTGGATGTC TCCTCCCCCA ATATTACCTG ATGTATCTTA CATATTGCCA

12121 GGCTGATATT TTAAGACATT AAAAGGTATA TTTCATTATT GAGCCACATG GTATTGATTA

12181 CTGCTTACTA AAATTTTGTC ATTGTACACA TCTGTAAAAG GTGGTTCCTT TTGGAATGCA

12241 AAGTTCAGGT GTTTGTTGTC TTTCCTGACC TAAGGTCTTG TGAGCTTGTA TTTTTTCTAT

12301 TTAAGCAGTG CTTTCTCTTG GACTGGCTTG ACTCATGGCA TTCTACACGT TATTGCTGGT

12361 CTAAATGTGA TTTTGCCAAG CTTCTTCAGG ACCTATAATT TTGCTTGACT TGTAGCCAAA

12421 CACAAGTAAA ATGATTAAGC AACAAATGTA TTTGTGAAGC TTGGTTTTTA GGTTGTTGTG

12481 TTGTGTGTGC TTGTGCTCTA TAATAATACT ATCCAGGGGC TGGAGAGGTG GCTCGGAGTT

12541 CAAGAGCACA GACTGCTCTT CCAGAAGTCC TGAGTTCAAT TCCCAGCAAC CACATGGTGG

12601 CTCACAACCA TCTGTAATGG GATCTGATGC CCTCTTCTGG TGTGTCTGAA GACCACAAGT

12661 GTATTCACAT TAAATAAATA AATCCTCCTT CTTCTTCTTT TTTTTTTTTT TAAAGAGAAT

12721 ACTGTCTCCA GTAGAATTTA CTGAAGTAAT GAAATACTTT GTGTTTGTTC CAATATGGTA

12781 GCCAATAATC AAATTACTCT TTAAGCACTG GAAATGTTAC CAAGGAACTA ATTTTTATTT

12841 GAAGTGTAAC TGTGGACAGA GGAGCCATAA CTGCAGACTT GTGGGATACA GAAGACCAAT

12901 GCAGACTTTA ATGTCTTTTC TCTTACACTA AGCAATAAAG AAATAAAAAT TGAACTTCTA

12961 GTATCCTATT TGTTTAAACT GCTAGCTTTA CTTAACTTTT GTGCTTCATC TATACAAAGC

13021 TGAAAGCTAA GTCTGCAGCC ATTACTAAAC ATGAAAGCAA GTAATGATAA TTTTGGATTT

13081 CAAAAATGTA GGGCCAGAGT TTAGCCAGCC AGTGGTGGTG CTTGCCTTTA TGCCTTTAAT

13141 CCCAGCACTC TGGAGGCAGA GACAGGCAGA TCTCTGAGTT TGAGCCCAGC CTGGTCTACA

13201 CATCAAGTTC TATCTAGGAT AGCCAGGAAT ACACACAGAA ACCCTGTTGG GGAGGGGGGC

13261 TCTGAGATTT CATAAAATTA TAATTGAAGC ATTCCCTAAT GAGCCACTAT GGATGTGGCT

13321 AAATCCGTCT ACCTTTCTGA TGAGATTTGG GTATTATTTT TTCTGTCTCT GCTGTTGGTT

13381 GGGTCTTTTG ACACTGTGGG CTTTCTTTAA AGCCTCCTTC CTGCCATGTG GTCTCTTGTT

13441 TGCTACTAAC TTCCCATGGC TTAAATGGCA TGGCTTTTTG CCTTCTAAGG GCAGCTGCTG

13501 AGATTTGCAG CCTGATTTCC AGGGTGGGGT TGGGAAATCT TTCAAACACT AAAATTGTCC

13561 TTTAATTTTT TTTTTAAAAA ATGGGTTATA TAATAAACCT CATAAAATAG TTATGAGGAG

13621 TGAGGTGGAC TAATATTAAA TGAGTCCCTC CCCTATAAAA GAGCTATTAA GGCTTTTTGT

13681 CTTATACTTA ACTTTTTTTT TAAATGTGGT ATCTTTAGAA CCAAGGGTCT TAGAGTTTTA

13741 GTATACAGAA ACTGTTGCAT CGCTTAATCA GATTTTCTAG TTTCAAATCC AGAGAATCCA

13801 AATTCTTCAC AGCCAAAGTC AAATTAAGAA TTTCTGACTT TTAATGTTAA TTTGCTTACT

13861 GTGAATATAA AAATGATAGC TTTTCCTGAG GCAGGGTCTC ACTATGTATC TCTGCCTGAT

13921 CTGCAACAAG ATATGTAGAC TAAAGTTCTG CCTGCTTTTG TCTCCTGAAT ACTAAGGTTA

13981 AAATGTAGTA ATACTTTTGG AACTTGCAGG TCAGATTCTT TTATAGGGGA CACACTAAGG

14041 GAGCTTGGGT GATAGTTGGT AAAATGTGTT TCAAGTGATG AAAACTTGAA TTATTATCAC

14101 CGCAACCTAC TTTTTAAAAA AAAAAGCCAG GCCTGTTAGA GCATGCTTAA GGGATCCCTA

14161 GGACTTGCTG AGCACACAAG AGTAGTTACT TGGCAGGCTC CTGGTGAGAG CATATTTCAA

14221 AAAACAAGGC AGACAACCAA GAAACTACAG TTAAGGCCGG CCGCGGTGGA GCTCCAGCTT

14281 TTGTTCCCTT TAGTGAGGGT TAATTTCGAG CTTGGCGTAA TCATGGTCAT AGCTGTTTCC

14341 TGTGTGAAAT TGTTATCCGC TCACAATTCC ACACAACATA CGAGCCGGAA GCATAAAGTG

14401 TAAAGCCTGG GGTGCCTAAT GAGTGAGCTA ACTCACATTA ATTGCGTTGC GCTCACTGCC

14461 CGCTTTCCAG TCGGGAAACC TGTCGTGCCA GCTGCATTAA TGAATCGGCC AACGCGCGGG

14521 GAGAGGCGGT TTGCGTATTG GGCGCTCTTC CGCTTCCTCG CTCACTGACT CGCTGCGCTC

14581 GGTCGTTCGG CTGCGGCGAG CGGTATCAGC TCACTCAAAG GCGGTAATAC GGTTATCCAC

14641 AGAATCAGGG GATAACGCAG GAAAGAACAT GTGAGCAAAA GGCCAGCAAA AGGCCAGGAA

14701 CCGTAAAAAG GCCGCGTTGC TGGCGTTTTT CCATAGGCTC CGCCCCCCTG ACGAGCATCA

14761 CAAAAATCGA CGCTCAAGTC AGAGGTGGCG AAACCCGACA GGACTATAAA GATACCAGGC

14821 GTTTCCCCCT GGAAGCTCCC TCGTGCGCTC TCCTGTTCCG ACCCTGCCGC TTACCGGATA

14881 CCTGTCCGCC TTTCTCCCTT CGGGAAGCGT GGCGCTTTCT CATAGCTCAC GCTGTAGGTA

14941 TCTCAGTTCG GTGTAGGTCG TTCGCTCCAA GCTGGGCTGT GTGCACGAAC CCCCCGTTCA

15001 GCCCGACCGC TGCGCCTTAT CCGGTAACTA TCGTCTTGAG TCCAACCCGG TAAGACACGA

15061 CTTATCGCCA CTGGCAGCAG CCACTGGTAA CAGGATTAGC AGAGCGAGGT ATGTAGGCGG

15121 TGCTACAGAG TTCTTGAAGT GGTGGCCTAA CTACGGCTAC ACTAGAAGGA CAGTATTTGG

15181 TATCTGCGCT CTGCTGAAGC CAGTTACCTT CGGAAAAAGA GTTGGTAGCT CTTGATCCGG

15241 CAAACAAACC ACCGCTGGTA GCGGTGGTTT TTTTGTTTGC AAGCAGCAGA TTACGCGCAG

15301 AAAAAAAGGA TCTCAAGAAG ATCCTTTGAT CTTTTCTACG GGGTCTGACG CTCAGTGGAA

15361 CGAAAACTCA CGTTAAGGGA TTTTGGTCAT GAGATTATCA AAAAGGATCT TCACCTAGAT

15421 CCTTTTAAAT TAAAAATGAA GTTTTAAATC AATCTAAAGT ATATATGAGT AAACTTGGTC

15481 TGACAGTTAC CAATGCTTAA TCAGTGAGGC ACCTATCTCA GCGATCTGTC TATTTCGTTC

15541 ATCCATAGTT GCCTGACTCC CCGTCGTGTA GATAACTACG ATACGGGAGG GCTTACCATC

15601 TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA CCGGCTCCAG ATTTATCAGC

15661 AATAAACCAG CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT CCTGCAACTT TATCCGCCTC

15721 CATCCAGTCT ATTAATTGTT GCCGGGAAGC TAGAGTAAGT AGTTCGCCAG TTAATAGTTT

15781 GCGCAACGTT GTTGCCATTG CTACAGGCAT CGTGGTGTCA CGCTCGTCGT TTGGTATGGC

15841 TTCATTCAGC TCCGGTTCCC AACGATCAAG GCGAGTTACA TGATCCCCCA TGTTGTGCAA

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15901 AAAAGCGGTT AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA AGTAAGTTGG CCGCAGTGTT

15961 ATCACTCATG GTTATGGCAG CACTGCATAA TTCTCTTACT GTCATGCCAT CCGTAAGATG

16021 CTTTTCTGTG ACTGGTGAGT ACTCAACCAA GTCATTCTGA GAATAGTGTA TGCGGCGACC

16081 GAGTTGCTCT TGCCCGGCGT CAATACGGGA TAATACCGCG CCACATAGCA GAACTTTAAA

16141 AGTGCTCATC ATTGGAAAAC GTTCTTCGGG GCGAAAACTC TCAAGGATCT TACCGCTGTT

16201 GAGATCCAGT TCGATGTAAC CCACTCGTGC ACCCAACTGA TCTTCAGCAT CTTTTACTTT

16261 CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT GCCGCAAAAA AGGGAATAAG

16321 GGCGACACGG AAATGTTGAA TACTCATACT CTTCCTTTTT CAATATTATT GAAGCATTTA

16381 TCAGGGTTAT TGTCTCATGA GCGGATACAT ATTTGAATGT ATTTAGAAAA ATAAACAAAT

16441 AGGGGTTCCG CGCACATTTC CCCGAAAAGT GCCACCTAAA TTGTAAGCGT TAATATTTTG

16501 TTAAAATTCG CGTTAAATTT TTGTTAAATC AGCTCATTTT TTAACCAATA GGCCGAAATC

16561 GGCAAAATCC CTGTTTAAAC TCGCGACGCG TTATAAATCA AAAGAATAGA CCGAGATAGG
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Supplementary material S3, details on the construct design, corresponding to the manuscript:

Ubiquitous transgenic overexpression of C-C chemokine ligand 2: a model to assess the combined effect of high energy intake and continuous low-grade inflammation?

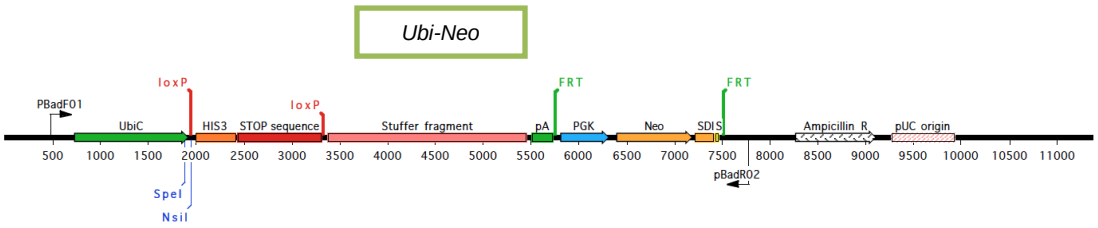
Esther Rodríguez-Gallego, Marta Riera-Borrull, Anna Hernández-Aguilera, Roger Mariné-Casadó, Anna Rull, Raúl Beltrán-Debón, Fedra Luciano-Mateo, Javier A. Menéndez[1], Alejandro Vazquez-Martin[1], Juan J. Sirvent[2], Vicente Martín-Paredero[3], Angel L. Corbí[4], Elena Sierra-Filardi[4], Gerard Aragonès, Anabel García-Heredia, Jordi Camps, Carlos Alonso-Villaverde[5], Jorge Joven*

The Knock-in Construct Design, from plasmid to electroporation

1. Overview

1.1 Vector backbone

The following plasmid (named *Ubi-Neo*) was used for construction of the *targeting vector*.



1.2 Construction fragments

Named *frag arm*, contained the genomic fragment of the gene to be introduced (*Ccl2*).

2. *frag arm* (Generation, Diagnostics and Sequencing)

2.1 Production

Using the following primers, the genomic fragment of *Ccl2* gene was amplified by PCR.

Template: C57BL/6 genomic DNA

P_01 5' *AGAATTC*AAAGCAGAGCCACTCCATTCACAC

EcoRI Homology

P_02 5' *TGAATTC*TCCCTCCCTCTTTATTGGACCGAAG

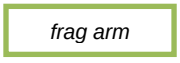
EcoRI Homology

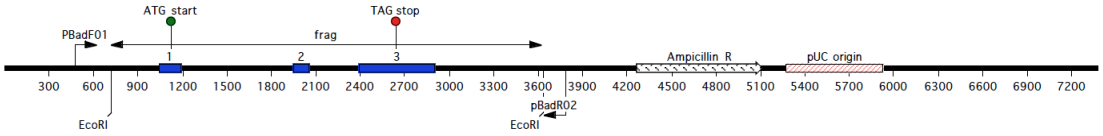
Product: 2921 bp

2.2 Cloning into pPCR

a) Sequencing with PBadF01 or PBadR02

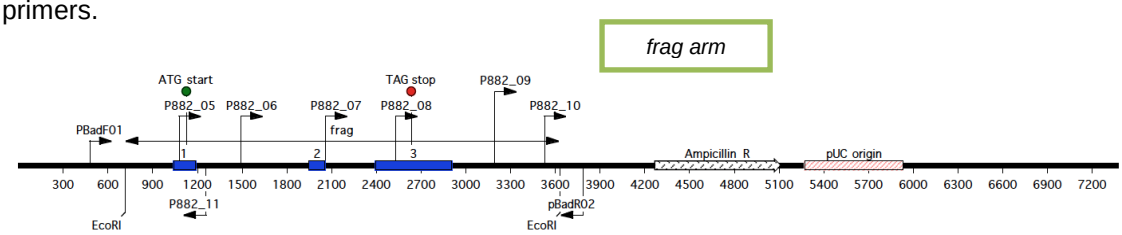
The PCR product was cloned into pPCR and screened by sequencing to confirm clones with the correct insert.





b) Full Sequencing

Once end sequencing identified clones, a full sequence was completed using the following primers.

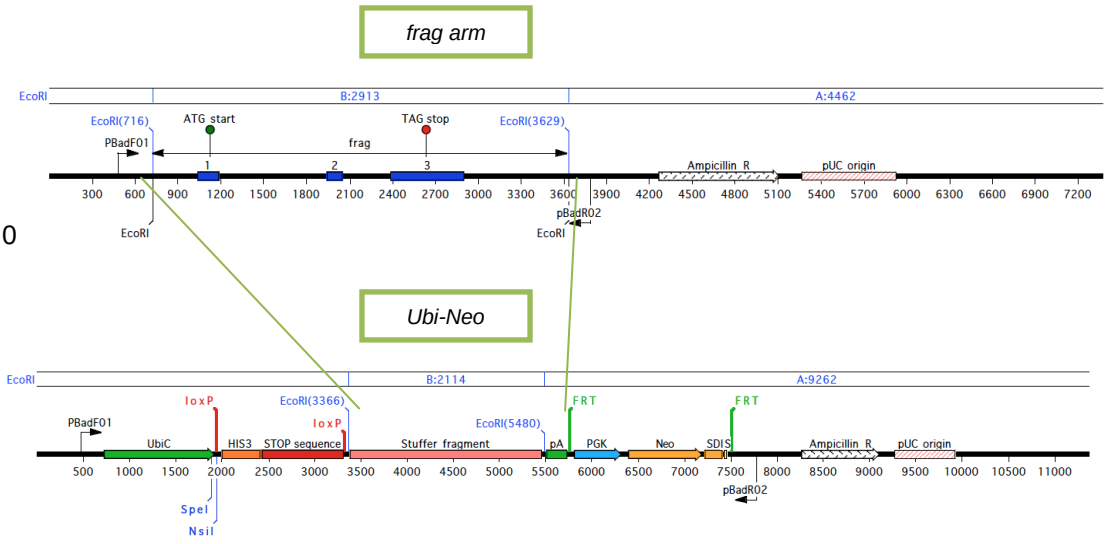


2.3 Cloning into the targeting vector

a) Restriction enzyme digest for cloning

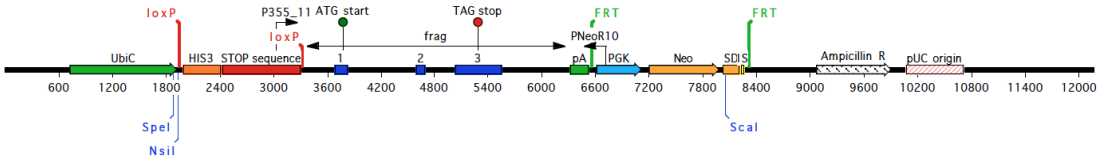
Both *frag arm* vector (containing Ccl2 gene) and the *Ubi-Neo* vector, were digested with EcoRI for cloning *Ccl2* gene into *Ubi-Neo* vector (just where the *Stuffer fragment* was placed).

The resulting vector was named *Ubi-frag-Neo*



b) Screen by Sequencing

Clones containing the insert could be screened by end sequencing with P335_11 and PNeoR10 primers:



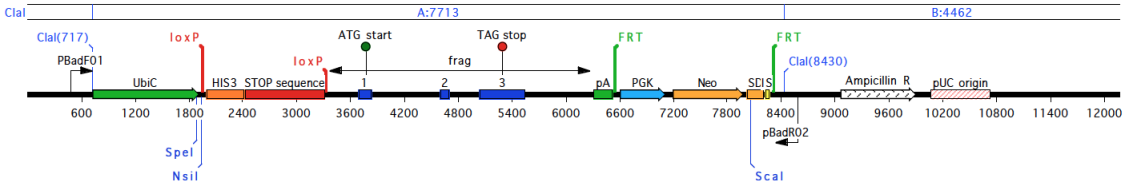
3. Cloning into the ROSA 26 vector

3.1 Cloning into the targeting vector

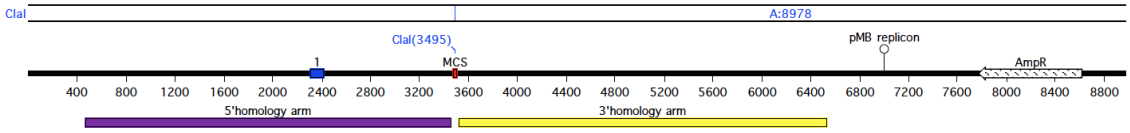
a) Restriction enzyme digest for cloning

ClaI restriction enzyme was used for cloning the constructed fragment (*Ubi-frag-Neo*) into the *ROSA26 vector* (which contained homologous sequences to ROSA26).

Ubi-frag-Neo

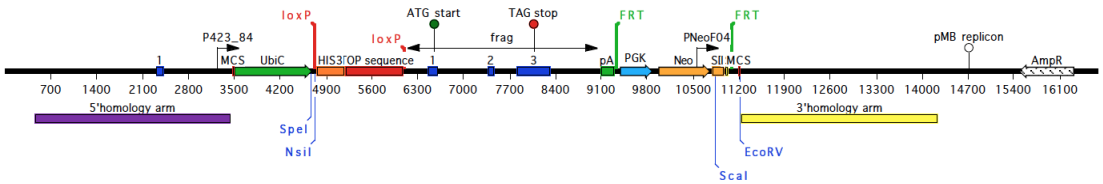


ROSA26 vector



b) Screen by Sequencing

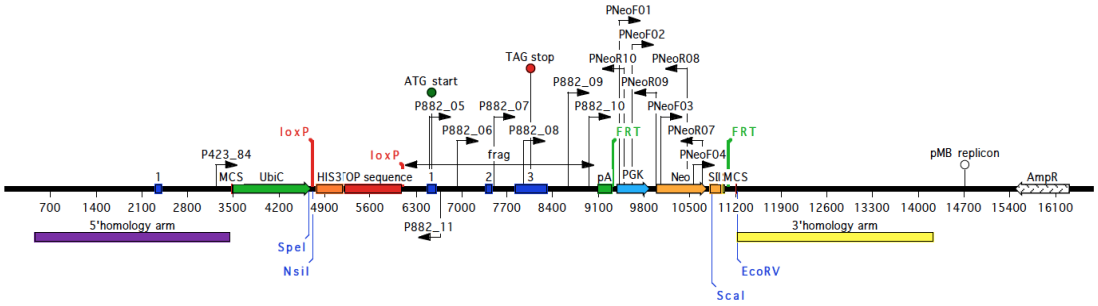
Clones that contained the insert (the *targeting vector*), were screened by end sequencing with the following primers



4. Complete *Targeting Vector*

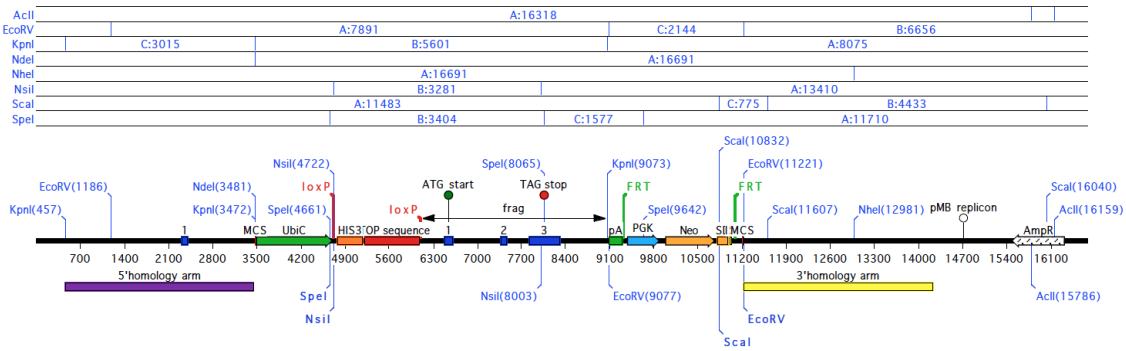
4.1 Final sequencing

To assess the *targeting vector* integrity, final sequencing was performed using the primers identified in the following graphic:



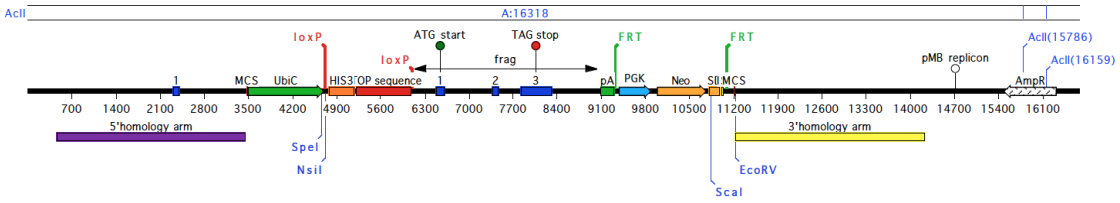
4.2 Restriction enzyme digest for Quality Control

Targeting vector was digested with several restriction enzymes as quality control strategy:



4.3 Restriction enzyme digest for electroporation

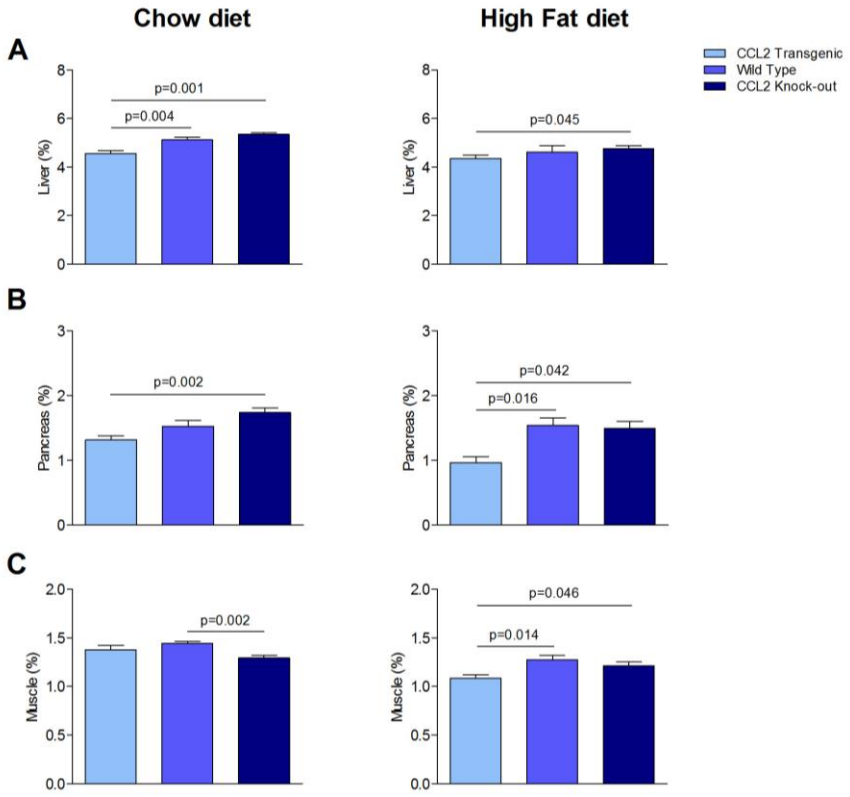
Finally, *targeting vector* was digested with AclI to obtain a suitable vector for electroporation into stem cells



Supplementary material S4, Weight of selected tissues and organs, corresponding to the manuscript:

Ubiquitous transgenic overexpression of C-C chemokine ligand 2: a model to assess the combined effect of high energy intake and continuous low-grade inflammation?

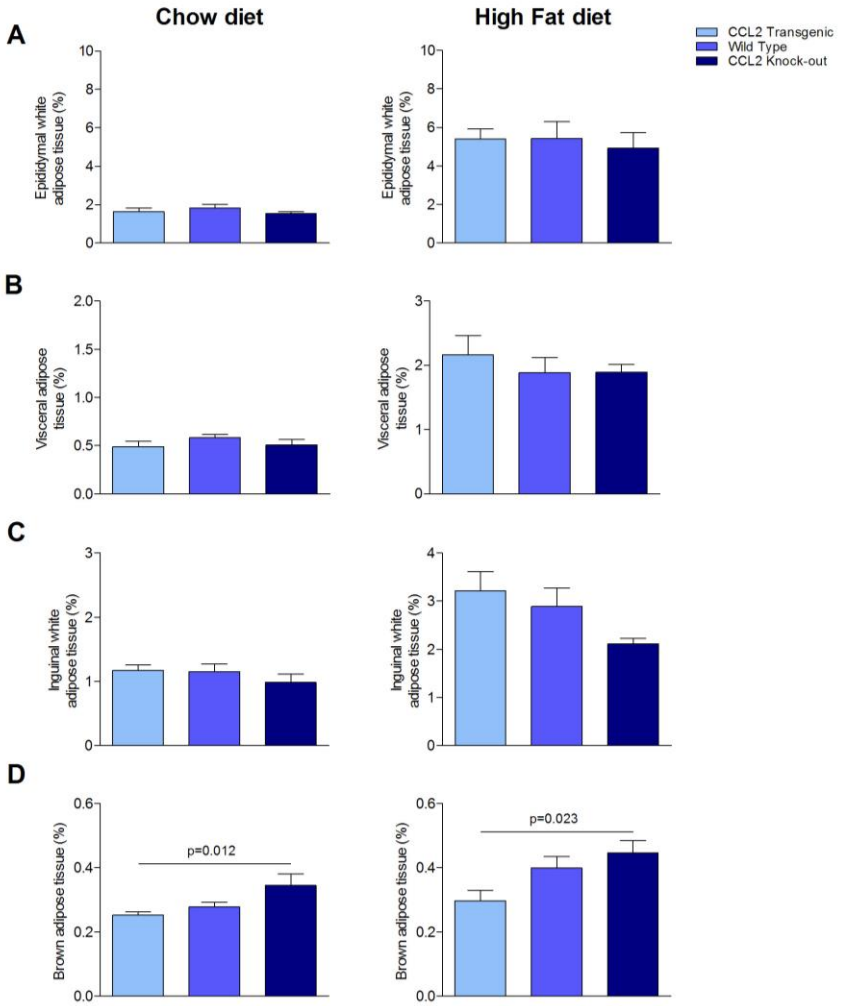
Esther Rodríguez-Gallego, Marta Riera-Borrull, Anna Hernández-Aguilera, Roger Mariné-Casadó, Anna Rull, Raúl Beltrán-Debón, Fedra Luciano-Mateo, Javier A. Menéndez[1], Alejandro Vazquez-Martin[1], Juan J. Sirvent[2], Vicente Martín-Paredero[3], Angel L. Corbí[4], Elena Sierra-Filardi[4], Gerard Aragonès, Anabel García-Heredia, Jordi Camps, Carlos Alonso-Villaverde[5], Jorge Joven*


Chow diet
High Fat diet
CCL2 Transgenic
Wild Type
CCL2 Knock-out
A
Liver (%)
p=0.001
p=0.004
p=0.045
B
Pancreas (%)
p=0.002
p=0.042
p=0.016
C
Muscle (%)
p=0.002
p=0.046
p=0.014

Supplementary material S5, Weight of adipose tissues in the three strains after 14 weeks of a chow diet or high fat diet treatment.

Ubiquitous transgenic overexpression of C-C chemokine ligand 2: a model to assess the combined effect of high energy intake and continuous low-grade inflammation?

Esther Rodríguez-Gallego, Marta Riera-Borrull, Anna Hernández-Aguilera, Roger Mariné-Casadó, Anna Rull, Raúl Beltrán-Debón, Fedra Luciano-Mateo, Javier A. Menéndez[1], Alejandro Vazquez-Martin[1], Juan J. Sirvent[2], Vicente Martín-Paredero[3], Angel L. Corbí[4], Elena Sierra-Filardi[4], Gerard Aragonès, Anabel García-Heredia, Jordi Camps, Carlos Alonso-Villaverde[5], Jorge Joven*

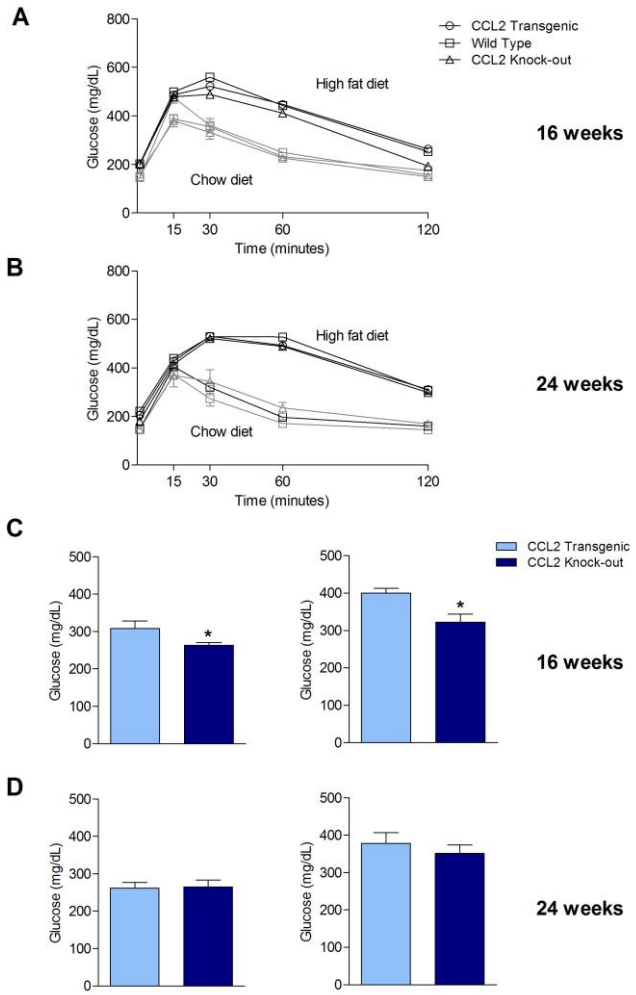


Supplementary material S6, disturbances in glucose metabolism, corresponding to the manuscript:

Ubiquitous transgenic overexpression of C-C chemokine ligand 2: a model to assess the combined effect of high energy intake and continuous low-grade inflammation?

Esther Rodríguez-Gallego, Marta Riera-Borrull, Anna Hernández-Aguilera, Roger Mariné-Casadó, Anna Rull, Raúl Beltrán-Debón, Fedra Luciano-Mateo, Javier A. Menéndez[1], Alejandro Vazquez-Martin[1], Juan J. Sirvent[2], Vicente Martín-Paredero[3], Angel L. Corbí[4], Elena Sierra-Filardi[4], Gerard Aragonès, Anabel García-Heredia, Jordi Camps, Carlos Alonso-Villaverde[5], Jorge Joven*

Oral glucose tolerance tests displayed a significant effect of high fat, high-cholesterol diet on insulin resistance even with a short-term manipulation. However, differences among strains fed the same diet were negligible either in 16 an 24 weeks old (A, B). Plasma glucose was higher in CCL2 overexpressors than in other strains and the effect of diet was also more evident (C). These differences disappeared when the dietary tratement was for 14 weeks (D).
* P<0.05 with respect to relevant pair.

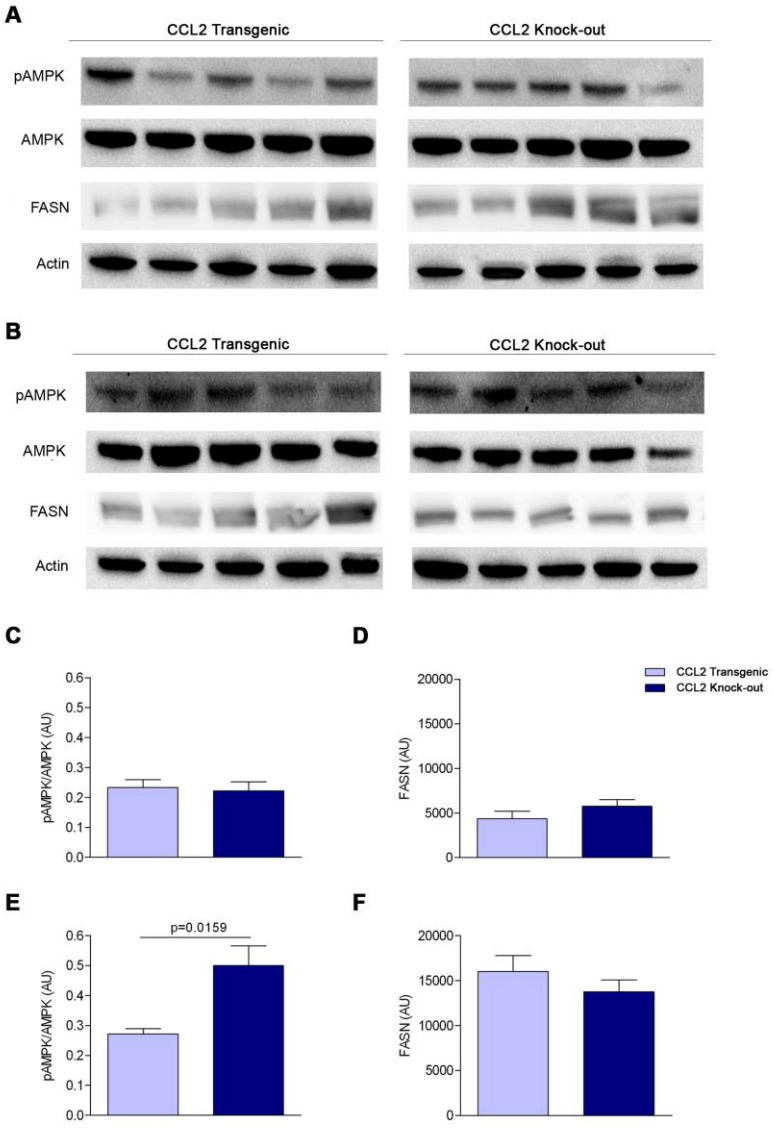


Supplementary material S7, expression of fatty acid synthase and AMPK in the liver, corresponding to the manuscript:

Ubiquitous transgenic overexpression of C-C chemokine ligand 2: a model to assess the combined effect of high energy intake and continuous low-grade inflammation?

Esther Rodríguez-Gallego, Marta Riera-Borrull, Anna Hernández-Aguilera, Roger Mariné-Casadó, Anna Rull, Raúl Beltrán-Debón, Fedra Luciano-Mateo, Javier A. Menéndez[1], Alejandro Vazquez-Martin[1], Juan J. Sirvent[2], Vicente Martín-Paredero[3], Angel L. Corbí[4], Elena Sierra-Filardi[4], Gerard Aragonès, Anabel García-Heredia, Jordi Camps, Carlos Alonso-Villaverde[5], Jorge Joven*

Representative immunoblottings in the liver of transgenic and KO mice fed chow diet (A,C,D) and high-fat diet (B,E, F) of FASN and activated AMPK and the respective calculations (C-F) .

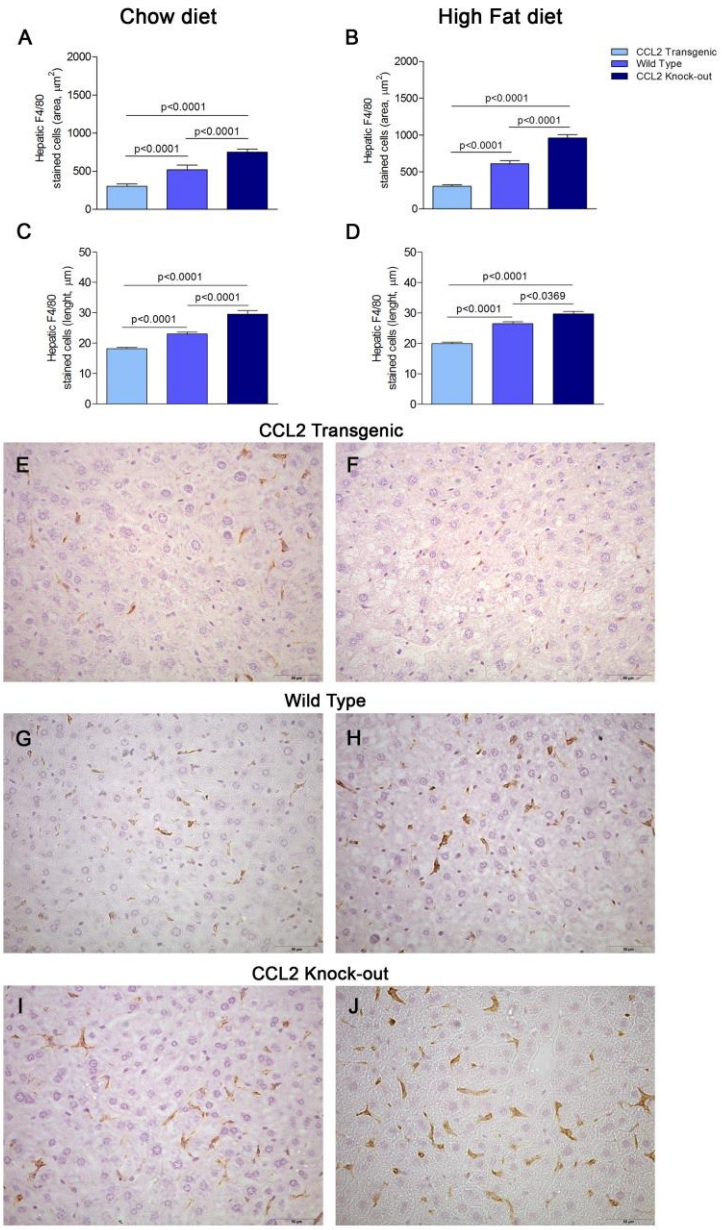

A
CCL2 Transgenic
CCL2 Knock-out
pAMPK
AMPK
FASN
Actin
B
CCL2 Transgenic
CCL2 Knock-out
pAMPK
AMPK
FASN
Actin
C
pAMPK/AMPK (AU)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
D
CCL2 Transgenic
CCL2 Knock-out
FASN (AU)
20000
15000
10000
5000
0
E
p=0.0159
pAMPK/AMPK (AU)
0.6
0.5
0.4
0.3
0.2
0.1
0.0
F
FASN (AU)
20000
15000
10000
5000
0

Supplementary material S8 dietary fat and overexpression of CCL2 modify the size, number and morphology of liver macrophages also after 14 weeks of dietary treatment corresponding to the manuscript:

Ubiquitous transgenic overexpression of C-C chemokine ligand 2: a model to assess the combined effect of high energy intake and continuous low-grade inflammation?

Esther Rodríguez-Gallego, Marta Riera-Borrull, Anna Hernández-Aguilera, Roger Mariné-Casadó, Anna Rull, Raúl Beltrán-Debón, Fedra Luciano-Mateo, Javier A. Menéndez[1], Alejandro Vazquez-Martin[1], Juan J. Sirvent[2], Vicente Martín-Paredero[3], Angel L. Corbí[4], Elena Sierra-Filardi[4], Gerard Aragonès, Anabel García-Heredia, Jordi Camps, Carlos Alonso-Villaverde[5], Jorge Joven*

Dietary fat (right column) and CCL2 expression modify the size, number and morphology of liver macrophages with respect to those fed a chow diet (left column) as assessed with F4/80 staining. Values for stained area and length of macrophages (A-D) are illustrated with representative microphotographs from transgenic (E,F), WT (G,H) and KO mice (I,J).

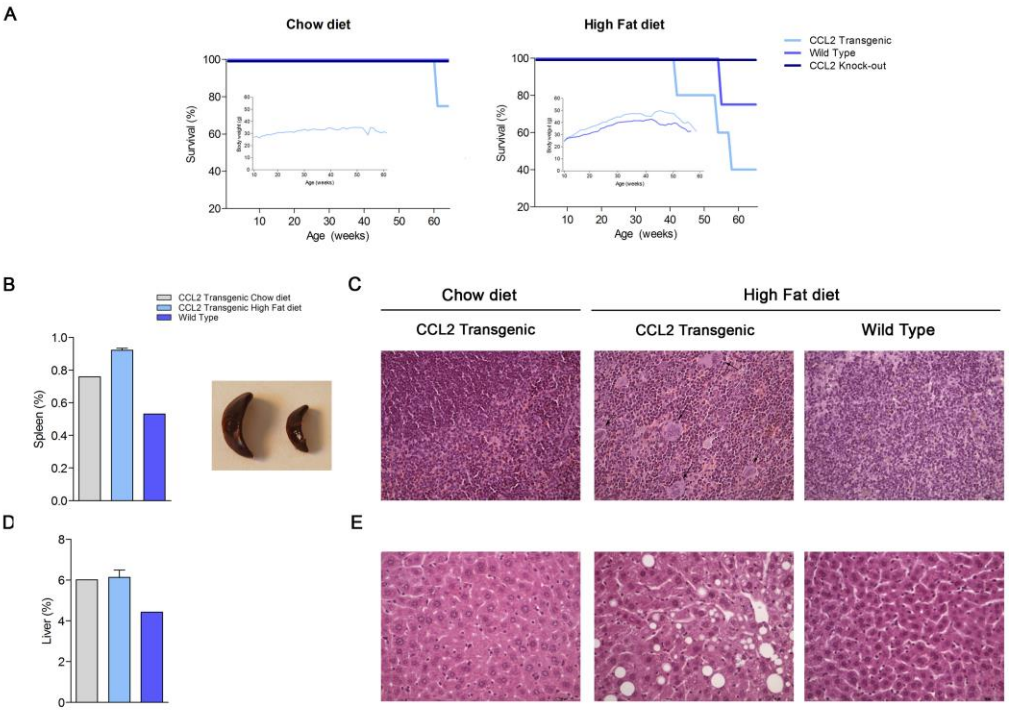

Chow diet
High Fat diet
A
B
C
D
CCL2 Transgenic
Wild Type
CCL2 Knock-out
Hepatic F4/80 stained cells (area, µm²)
Hepatic F4/80 stained cells (lenght, µm)
p<0.0001
p<0.0001
p<0.0001
p<0.0001
p<0.0001
p<0.0001
p<0.0001
p<0.0369
CCL2 Transgenic
E
F
Wild Type
G
H
CCL2 Knock-out
I
J

Supplementary material S9, Transgenic mice that overexpress CCL2 die prematurely when fed high-fat diet, corresponding to the manuscript:

Ubiquitous transgenic overexpression of C-C chemokine ligand 2: a model to assess the combined effect of high energy intake and continuous low-grade inflammation?

Esther Rodríguez-Gallego, Marta Riera-Borrull, Anna Hernández-Aguilera, Roger Mariné-Casadó, Anna Rull, Raúl Beltrán-Debón, Fedra Luciano-Mateo, Javier A. Menéndez[1], Alejandro Vazquez-Martin[1], Juan J. Sirvent[2], Vicente Martín-Paredero[3], Angel L. Corbí[4], Elena Sierra-Filardi[4], Gerard Aragonès, Anabel García-Heredia, Jordi Camps, Carlos Alonso-Villaverde[5], Jorge Joven*

Transgenic mice fed high-fat diet died prematurely after a progressive decrease in weight and activity (A,B). The size of the spleen in transgenic animals was higher than in controls and there were abundant megakariocytes (C, D). The livers were also higher in transgenic mice where steatosis and signs of regenerative tissue and apoptotic nuclei were numerous (E,F)


A
Chow diet
High Fat diet
CCL2 Transgenic
Wild Type
CCL2 Knock-out
Survival (%)
Age (weeks)
B
CCL2 Transgenic Chow diet
CCL2 Transgenic High Fat diet
Wild Type
Spleen (%)
C
Chow diet
High Fat diet
CCL2 Transgenic
CCL2 Transgenic
Wild Type
D
Liver (%)
E