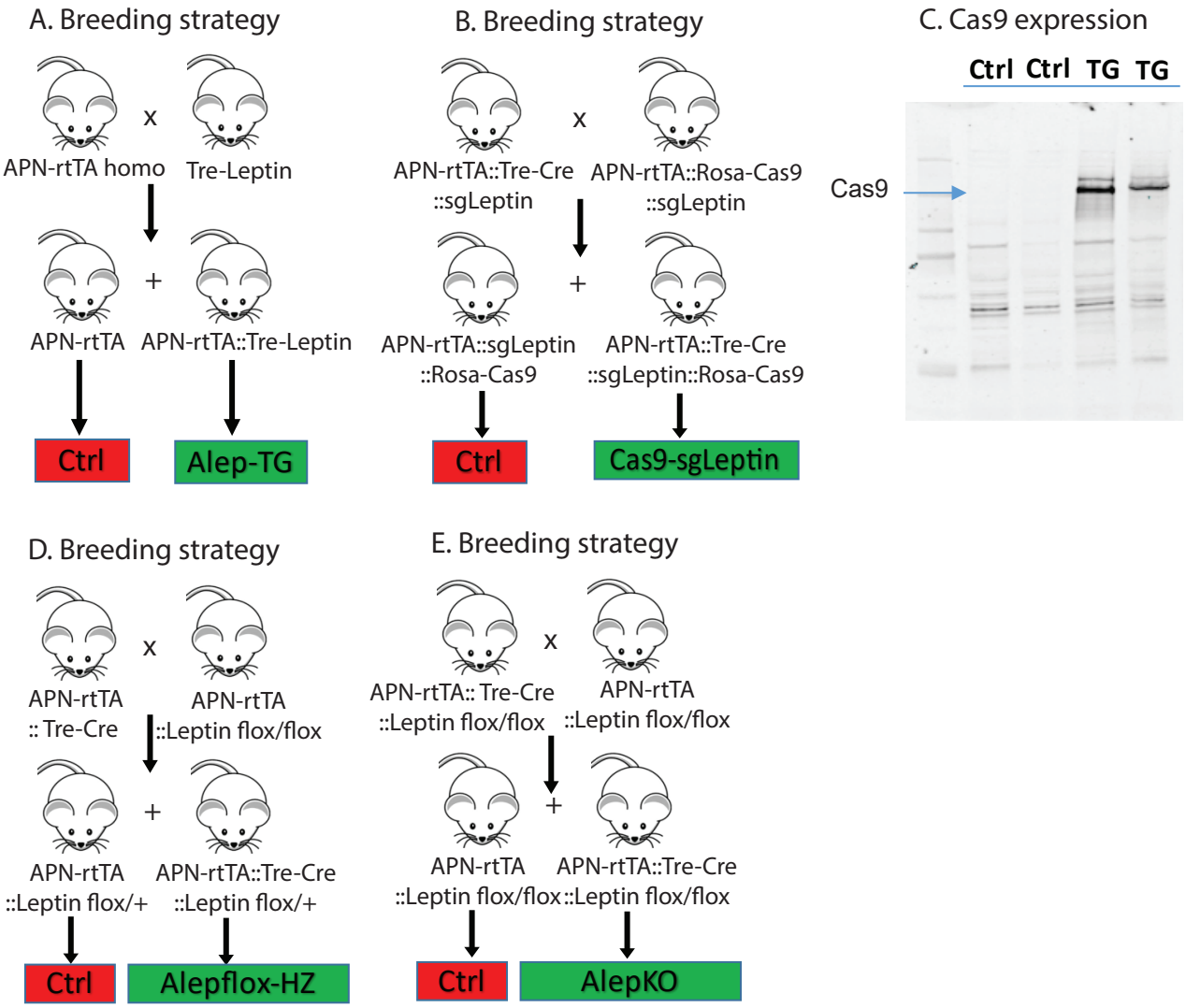
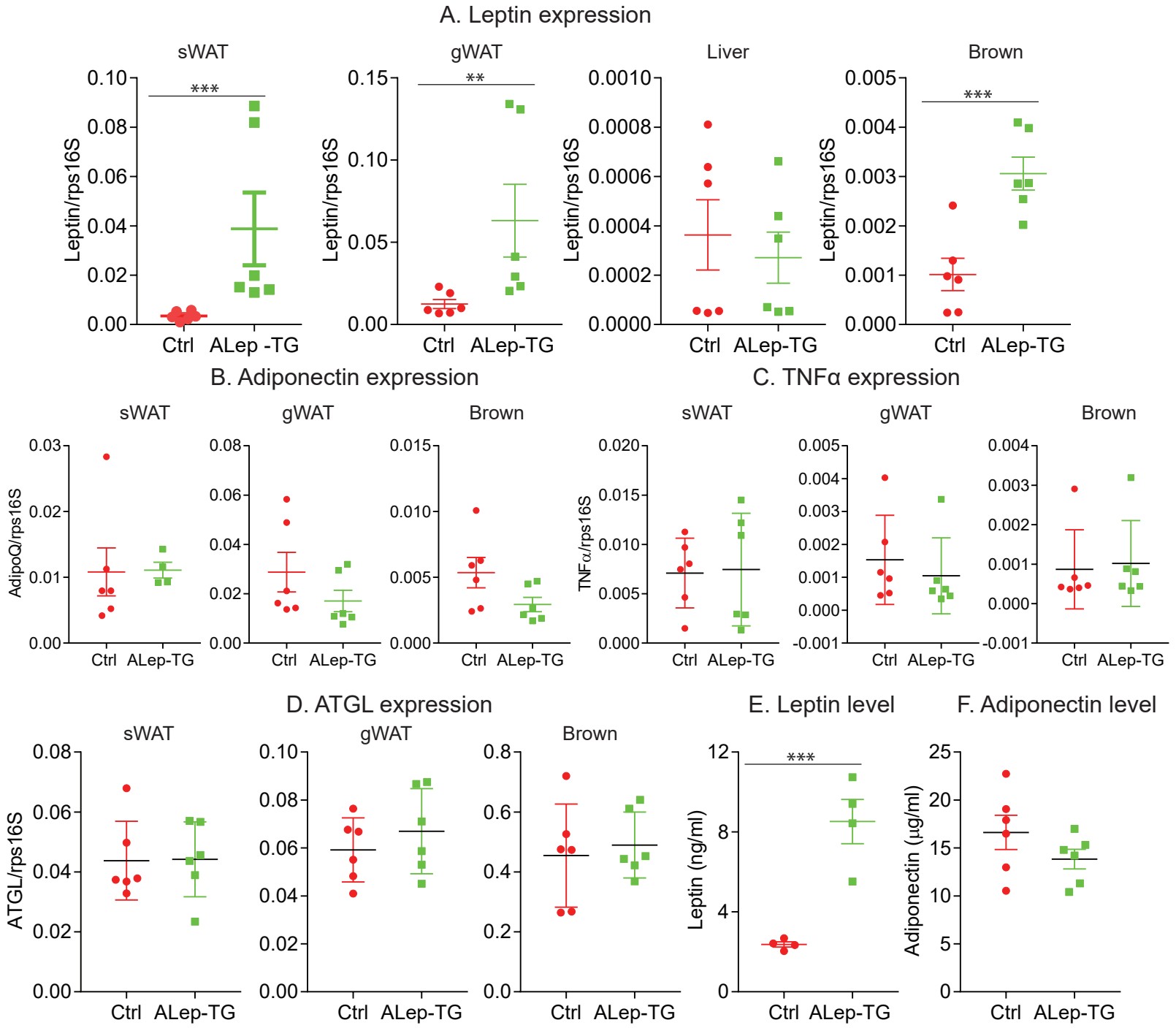


<u>Suppl. Figure 1:</u> Breeding strategy and genotyping for the mice used in this study.

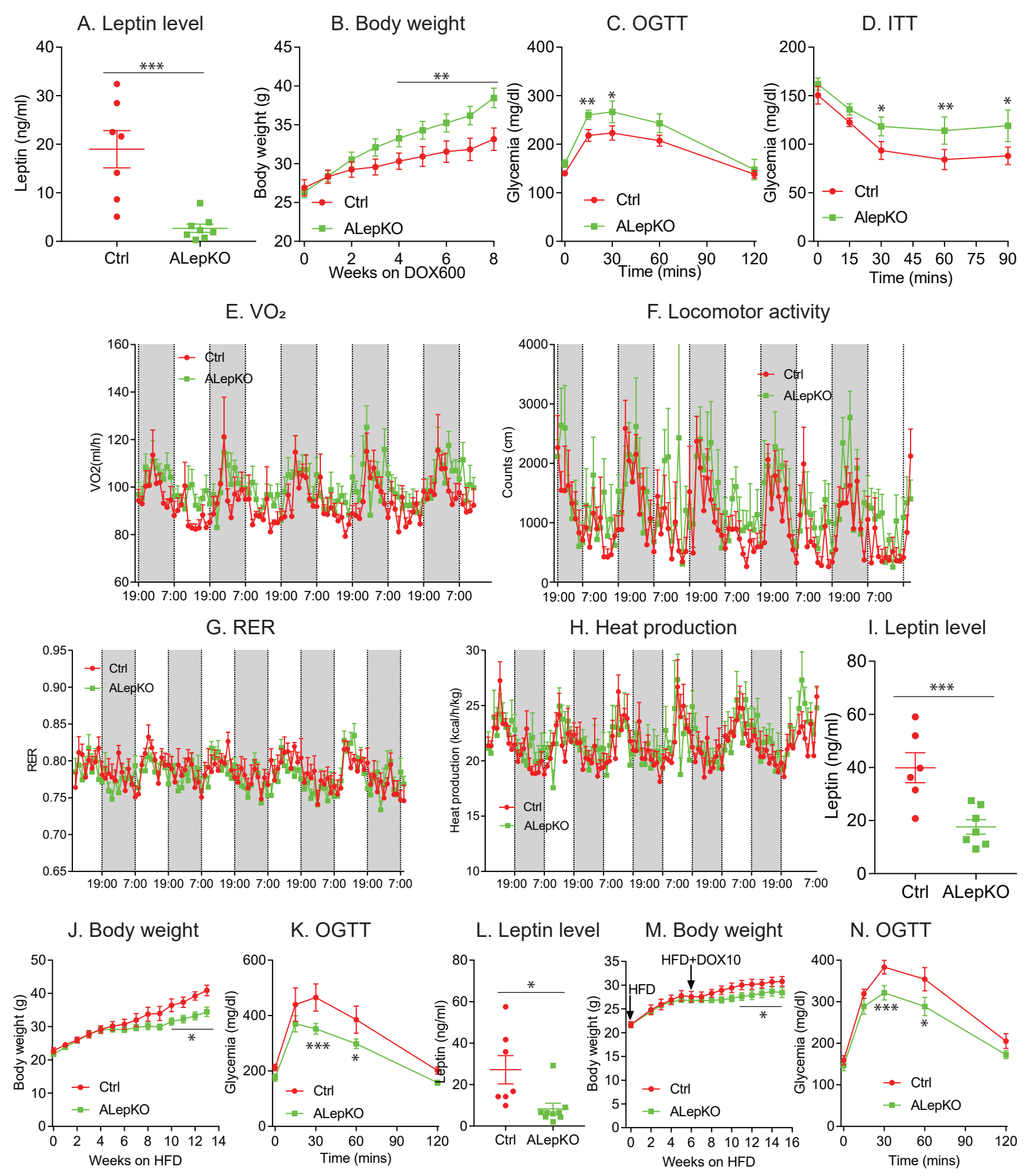
***Related to Star Methods*.**

(**A**) Breeding strategy for Alep-TG mice; (**B**) Breeding strategy for Cas9-sgLeptin mice; (**C**) Verification of Cas9 expression in sWAT; (**D**) Breeding strategy of ALepflox-HZ mice; (**E**) Breeding strategy of ALepKO mice.



<u>Suppl. Figure 2:</u> Verification of Alep mice. *Related to Figure 1*.

8-week old Alep-TG (*n* = 6) and littermate controls (*n* = 6) mice were placed on chow diet with DOX600 for one week. Then the mice were euthanized and various tissues (three fat depots and liver) were collected to measure *leptin* **(A),** *adiponectin* **(B)**, *TNFα* **(C)**, and *ATGL* **(D)** expression; also, plasma was taken for measuring leptin and adiponectin levels **(E)** circulating leptin levels; **(F)** circulating adiponectin levels. (Data are given as mean ± SEM. Error bars indicate SEM. **p* < 0.05; ***p* < 0.01; ****p* < 0.001).


A. Leptin level
Leptin (ng/ml)
Ctrl
ALepKO
B. Body weight
Body weight (g)
Weeks on DOX600
C. OGTT
Glycemia (mg/dl)
Time (mins)
D. ITT
E. VO₂
VO2(ml/h)
F. Locomotor activity
Counts (cm)
G. RER
RER
H. Heat production
Heat production (kcal/h/kg)
I. Leptin level
J. Body weight
Weeks on HFD
K. OGTT
L. Leptin level
M. Body weight
HFD
HFD+DOX10
N. OGTT

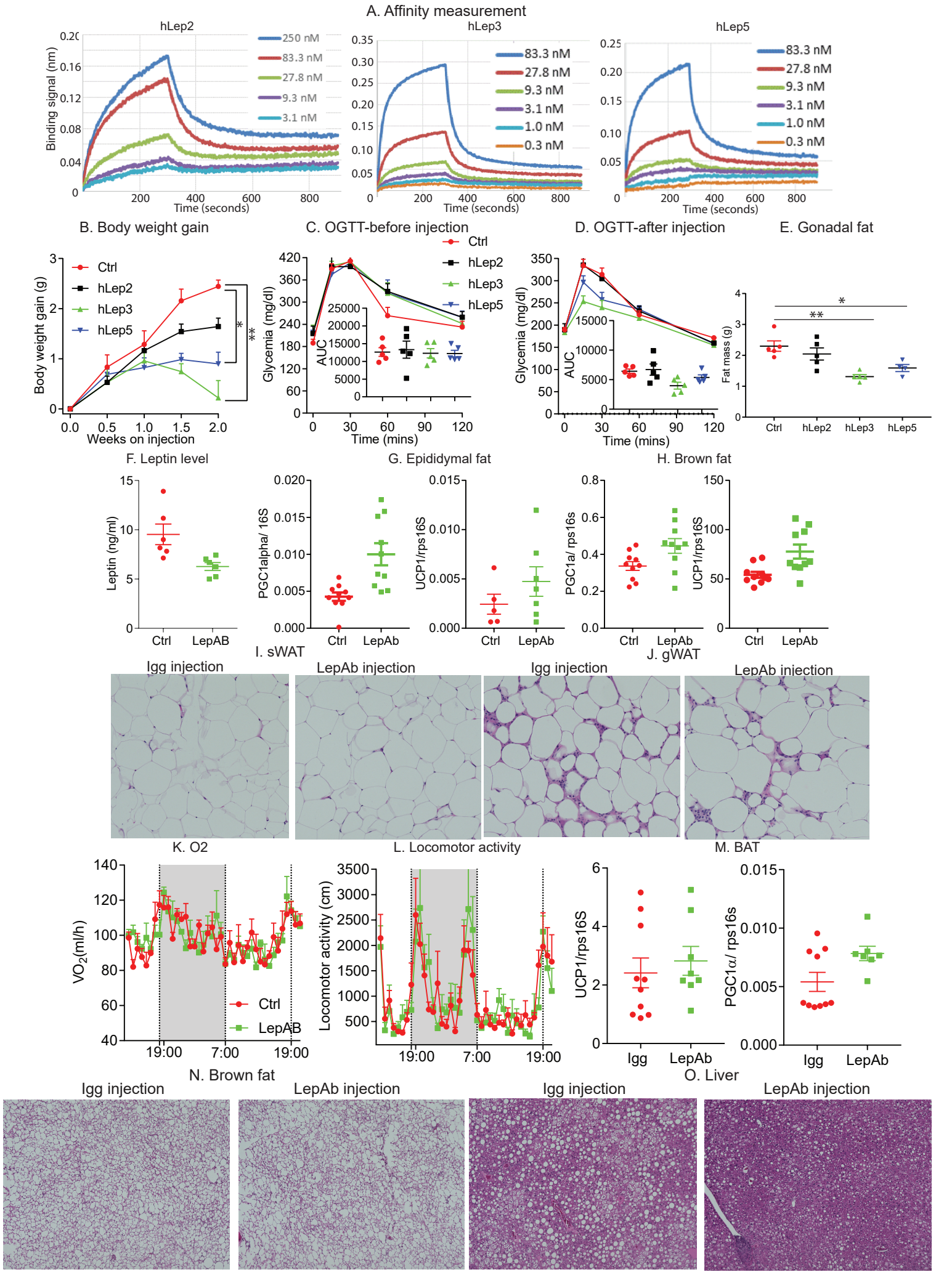
<u>Suppl. Figure 3:</u> A partial reduction of leptin slows down body weight gain and improves glucose tolerance. *Related to Figure 3*.

A cohort of AlepKO mice (*n* = 8) and littermate control mice (*n* = 7) were placed on chow diet with DOX600 for various time points, as indicated in the figures, leptin levels **(A)**, body weight **(B)**, OGTT **(C)** and ITT **(D)** were measured after 8 weeks.

A cohort of AlepKO mice (*n* = 6) and littermate control mice (*n* = 6) were placed into metabolic cages. Various parameters were measured; **(E)** traces of O_2 consumption and **(F)** locomotor activity, **(G)** RER and **(H)** heat production;

A cohort of AlepKO mice (*n* = 5) and littermate control (*n* = 5) mice were placed on chow diet with DOX600 for one week to induce some degree of leptin deletion, and then switched to HFD without Dox for different times. During the HFD period, body weights **(J)** were monitored on a weekly basis and OGTT **(K)** and leptin levels **(I)** were performed after 8-weeks on HFD.

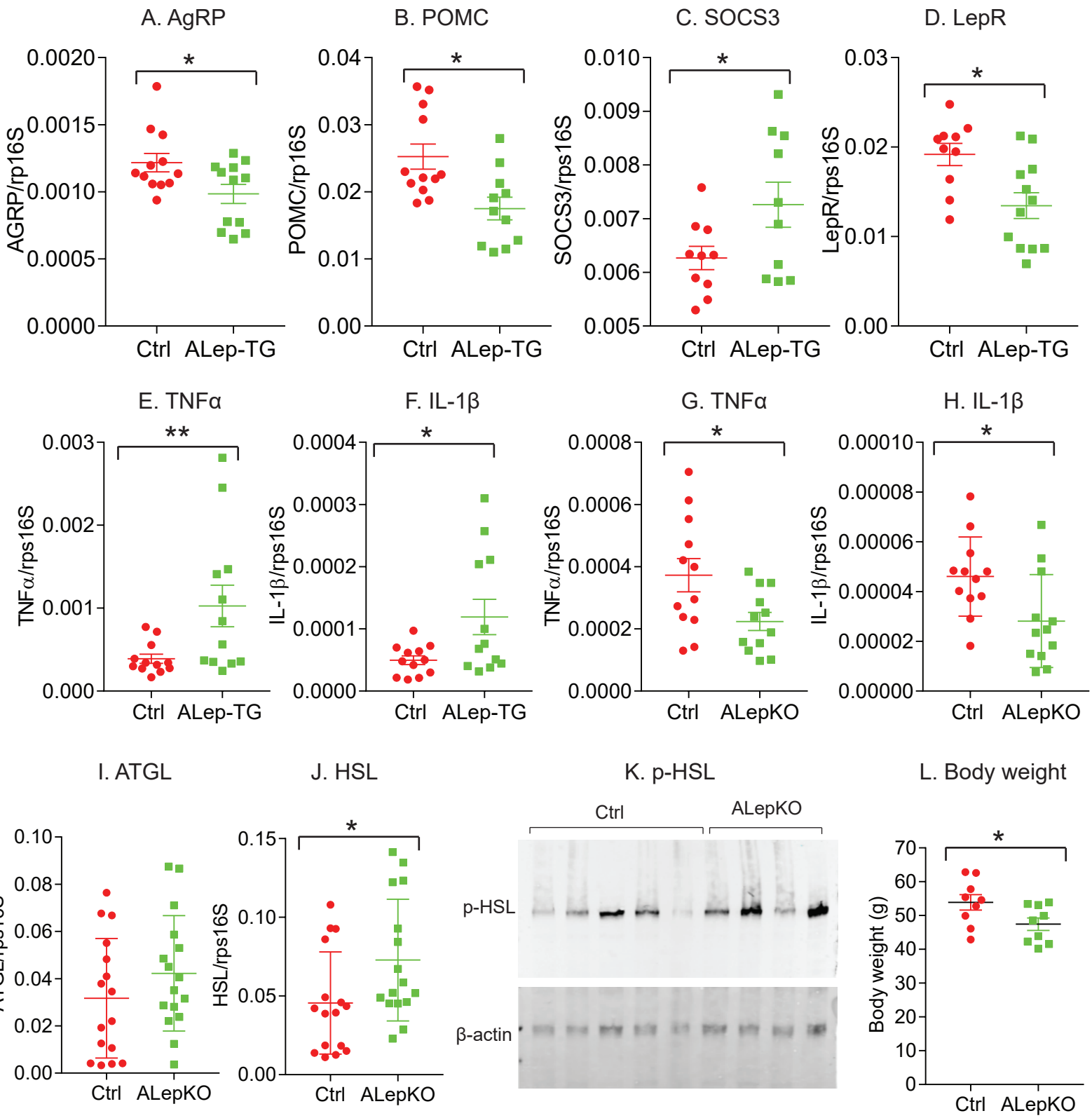
A cohort of AlepKO mice (*n* = 7) and littermate control (*n* = 7) mice were placed on HFD diet for 5 weeks and then switched to HFD plus DOX10 for another 8 weeks. Circulating leptin levels **(L)**, body weight **(M)** and OGTT **(N)** were measured after 8 weeks with DOX10. (Data are given as mean ± SEM. Error bars indicate SEM. *$p < 0.05$;**$p < 0.01$; ***$p < 0.001$).


A. Affinity measurement
hLep2
hLep3
hLep5
Binding signal (nm)
Time (seconds)
250 nM
83.3 nM
27.8 nM
9.3 nM
3.1 nM
1.0 nM
0.3 nM
B. Body weight gain
Body weight gain (g)
Weeks on injection
Ctrl
hLep2
hLep3
hLep5
C. OGTT-before injection
Glycemia (mg/dl)
Time (mins)
AUC
D. OGTT-after injection
E. Gonadal fat
Fat mass (g)
F. Leptin level
Leptin (ng/ml)
G. Epididymal fat
H. Brown fat
PGC1alpha/ 16S
UCP1/rps16S
PGC1a/ rps16s
I. sWAT
J. gWAT
Igg injection
LepAb injection
K. O2
VO2(ml/h)
L. Locomotor activity
Locomotor activity (cm)
M. BAT
N. Brown fat
O. Liver
Ctrl
LepAB
LepAb
Igg

<u>Suppl. Figure 4:</u> Leptin neutralizing antibodies reduce the extent of weight gain. ***Related to Figure 4*.**

(A) *In vitro* cell based assay for characterizing of three hLep neutralizing antibodies; Obese WT mice (*n* = 5 per group) were treated with vehicle or three different human leptin neutralizing antibodies for two weeks; **(B)** Effects of three different neutralizing antibodies (hLep2, hLep3 and hLep5) on body weight gain. OGTT before **(C)** and after **(D)** treatment with three different neutralizing antibodies; **(E)** Weight of epididymal adipose tissue after neutralizing antibody treatment; **(F)** free leptin levels after neutralizing antibody treatment; PGC1α and UCP1 expression in inguinal **(G)** and brown fat **(H)**; Histology of subQ **(I)** and epididymal fat **(J)** after neutralizing antibody treatment; Tracers of O_2 consumption **(K)** and locomotor activity **(L)** after neutralizing antibody treatment;

A cohort of obese WT mice (*n* = 5 per group) were housed in thermal chambers, and treated with control antibody (hIGG) and hLep3 antibody for 2 weeks. Then the mice were euthanized and tissues were collected for gene expression and histology. UCP1 and PGC1α expression **(M)** in brown fat were measured by RT-PCR. H&E staining of brown fat **(N)** and liver **(O)** under thermoneutral housing. **p* < 0.05; ***p* < 0.01; ****p* < 0.001.



<u>Suppl. Figure 5:</u> Partial reduction of leptin ameliorates ARH inflammation and increases adipose tissue leptin sensitivity. *Related to Figure 5.*

Expression levels of *agrp* **(A)**, *pomc* **(B)**, *socs3* **(C)**, *lepr* **(D),** *tnfα* **(E)** and *il-1β* **(F)** in ALep-TG and littermate Ctrl mice. TNFα **(G)** and IL-1β **(H)** in Ctrl and AlepKO mice; Expression of *atgl* **(I)** and *hsl* **(J)** in gonadal fat in AlepKO mice and littermate controls on HFD with Dox 10; **(K)** p-HSL level in gonadal fat of AlepKO and littermate control mice on HFD with DOX10. (**L**) Body weights of control and AlepKO mice after 8 months on HFD with Dox 10. **p* < 0.05; ***p* < 0.01; ****p* < 0.001.

Supplemental Table 1: List of primers and sequences for RT-PCR. Related to Star Methods

Gene name	Forward Primer	Reverse Primer
Atgl	GGATGGCGGCATTTCAGACA	CAAAGGGTTGGGTTGGTTCAG
Leptin	GAGACCCCTGTGTCGGTTC	CTGCGTGTGTGAAATGTCATTG
Adiponectin	TGTTCCTCTTAATCCTGCCCA	CCAACCTGCACAAGTTCCCTT
Ucp1	AGGCTTCCAGTACCATTAGGT	CTGAGTGAGGCAAAGCTGATTT
Pgc1α	TATGGAGTGACATAGAGTGTGCT	CCACTTCAATCCACCCAGAAAG
TNFα	GACGTGGAACTGGCAGAAGAG	TTGGTGGTTTGTGAGTGTGAG
Pomc	ATGCCGAGATTCTGCTACAGT	TCCAGCGAGAGGTCGAGTTT
Agrp	ATGCTGACTGCAATGTTGCTG	CAGACTTAGACCTGGGAACTCT
Socs3	ATGGTCACCCACAGCAAGTTT	TCCAGTAGAATCCGCTCTCCT
Lepr	TGGTCCCAGCAGCTATGGT	ACCCAGAGAAGTTAGCACTGT
IL-1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
Hsl	CCAGCCTGAGGGCTTACTG	CTCCATTGACTGTGACATCTCG
Atgl	GGATGGCGGCATTTCAGACA	CAAAGGGTTGGGTTGGTTCAG
Rps16s	CACTGCAAACGGGGAAATGG	CACCAGCAAATCGCTCCTTG

Supplemental Table 2: List of genotyping sequences. Related to Star Methods

Mouse strain	Forward Primer	Reverse Primer
Apn-rtTA	TGCAGGTCCTGATTGGATGTG	TTTCCTTGTCGTCAGGCCTTC
Tre-cre	GATTTCGACCAGGTTCGTTC	GCTAACCAGCGTTTTCGTTC
Rosa-rtTA	GAG TTCTCTGCTGCCTCCTG	CGAGGCGGATACAAGCAATA
sgLeptin	GGGCCTATTCCCATGAT	CGAAATACTTTCAAGTTACGGTAAGCA
Tre-Leptin	TCCACGCTGTTTGACCTCCA	TGAGGGTTTTGGTGTCATCCTG
Leptin flox	TGAGCAGTTCTGCAAACCAGCCT	AAGGGATGACTGTTCTGTGACTGC