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# FTO

## A Gene Contributing to Human Obesity

The *Fto* gene was first cloned after identification of a Fused toe (Ft) mutant mouse, whose phenotype arised from a 1.6mb deletion of six genes, including *Fto* [1, 2]. *FTO* is a very large gene, also known as fat mass and obesity associated gene. In human, it is located on chromosome 16, consisting of 9 exons and spanning more than 400kb [3]. *FTO* mRNA is widely expressed in different tissues, especially in the brain, but also in skeletal muscles and adipose tissue [3, 4, 5, 6]. In the mice brain, *Fto* is highly expressed in hypothalamic nuclei that control eating behavior [7, 8]. It was believed, that only vertebrates are carriers of the *FTO/Fto* gene. To date, also *FTO* homologs in evolutionary diverse marine eukaryotic algae were identified. The biological roles of these *FTO* homologs are still unknown [9].

Four regions in the *FTO* gene are particularly well conserved and three of them are homologous to *E. coli* AlkB and its eukaryotic homologs, members of the ABH (AlkB homolog) family [4]. AlkB is a member of the 2OG-FE(II) oxygenase superfamiliy that oxidatively demethylates DNA [4, 10]. 2OG-FE(II) oxygenases are involved in diverse processes, such as DNA repair, fatty acid metabolism and posttranslational modifications. *In vitro* studies have shown, that FTO can catalyze the demethylation of 3-methyluracil in single-stranded RNA with a slightly higher efficiency over that of 3-methylthymine in double- or single-stranded DNA [11]. Crystal structure analysis of the FTO protein provides a structural basis for the substrate specificity and the ability of FTO to distinguish 3-methyluracil and 3-methyl-

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thymine from other nucleotides [12]. A recent study has shown that FTO is a transcriptional coactivator for the C/EBP family of transcriptional regulators from unmethylated as well as methylated promoters and exhibits nuclease activity. Thus, FTO may play a role in the epigenetic regulation of fat tissue development and maintenance [13].

Based on twin studies, it was known for years, that the development of obesity in response to a particular environment underlies some genetic factors [14]. Some specific *FTO* gene variations were shown to correlate directly with obesity and even more, with type-II diabetes (T2D). The first intron of the *FTO* gene contains several SNPs (single nucleotide polymorphisms). 10 different SNPs correlating with an increased obesity risk have been identified within this intron [7]. The SNP rs9939609 A-allele for example increases the risk of obesity and T2D. Gathered data show that the effects on obesity are allelic dose dependent [7, 15]. Within the *FTO* gene, rs9930506 showed the strongest association with BMI (Body Mass Index), hip circumference, and weight [16]. These findings were reproduced for many Caucasian populations. Initial studies in Asians and lately also with an African population showed no association between *FTO* gene variants and the BMI or obesity [17-20]. However, other studies with Chinese, Koreans, Malay, Japanese, Europeans, Americans, and Hispanic Americans supported the association between *FTO* gene variants and obesity in such populations [16,19, 21-25]. Even though there is an association between *FTO* gene variants and the susceptibility to obesity, this can be overcome in part by physical activity [26, 27].

Ubiquitous overexpression of *FTO* in mice results in increased food intake and leads to a dose-dependent increase in obesity [28-30].

Inactivation of the *Fto* gene protects mice from obesity. The generated *Fto*-null mice lead to postnatal growth retardation and a significant reduction in adipose tissue and lean body mass [31]. However, the induction of hypothalamus development seems to be normal. The leanness of *Fto*-deficient mice results from an increased energy expenditure in the presence of reduced spontaneous locomotor activity with a relative hyperphagia. Interestingly, human carriers of the *FTO* gene risk allele seem to develop obesity as a consequence of hyperphagia, without altered energy expenditure as observed in mice [31]. The results are so far the first direct evidence of the energy homeostasis function of FTO. In contrast to *Fto*-deficient mice that, apart from the leanness phenotype, only show postnatal growth retartation, a loss-of-function mutation in the human *FTO* gene has been shown to cause a severe polymalformation syndrome. This indicates that FTO, apart from correlating with obesity, also is essential for normal development in human and might have different roles in different tissues [32, 33].

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Purified (PF) = Purified (Preservative free); FACS = Flow Cytometry; ICC = Immunocytochemistry; IP = Immunoprecipitation; IHC = Immunohistochemistry (FS = Frozen Sections, PS = Paraffin Sections); WB = Western blot; BP = Blocking Peptide

# NEW
# mAb AG103 – A Powerful Tool for FTO Studies

## new anti-FTO (human), mAb (AG103)

AG-20A-0092-C050 50 µg
AG-20A-0092-C100 100 µg

**CLONE:** AG103. **ISOTYPE:** Mouse IgG2aκ. **IMMUNOGEN:** Recombinant human FTO. **SPECIFICITY:** Recognizes human FTO. **APPLICATION:** IHC (PS), IP, WB.

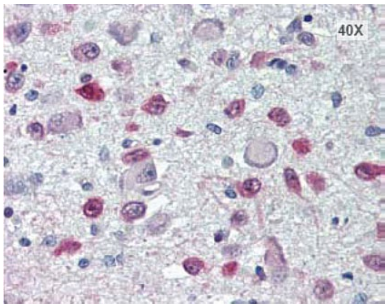



**FIGURE:** Immunohistochemical staining of FTO with anti-FTO (human), mAb (AG103) (Prod. No. AG-20A-0092) in brain, hypothalamus and paraventricular nucleus (5~10 µg/ml).

This antibody has been tested in immunohistochemistry, analyzed by an anatomic pathologist and validated for use in IHC applications against formalin-fixed, paraffin-embedded human tissues. The image shows the localization of the antibody as the precipitated red signal, with a hematoxylin purple nuclear counterstain (40x).

**FIGURE:** Western blot using human cell lysates.

1. HEK293T (100µg)
2. HEK293E (100µg)
3. HepG2 (100µg)
4. Hep3B (50µg)
5. V1T1 (100µg)
6. THP1 (100µg)
7. Molt4 (100µg)
8. A549 (50µg)

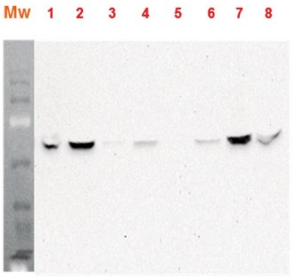
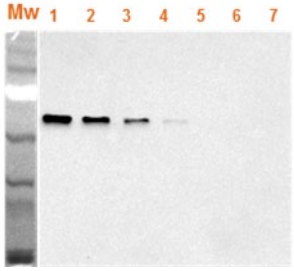
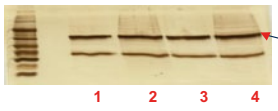



**FIGURE:** Immunoprecipitation.

1. Molt4 1mg
2. Molt4 500µg
3. Molt4 250µg
4. Molt4 125µg
5. Molt4 62.5µg
6. Molt4 31.2µg
7. Molt4 15.6µg

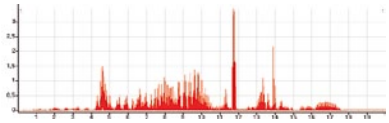



**A. IP & silver staining**

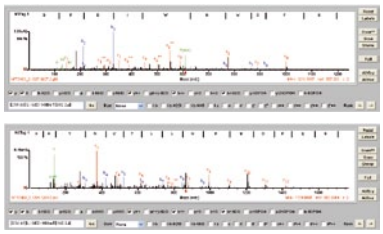



1. IP ; IgG control + RIPA control
2. IP ; IgG control + Molt4 1mg
3. IP ; AG103 + RIPA control
4. IP ; AG103 + Molt4 1mg

**B. Base peak chromatogram of in-gel digests**



**C. MS-MS spectrum**




**D. Protein sequence coverage (21%)**

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  1 MKRTPTAEER EREAKKLRLL EELEDTWLPY LTPKDDEFYQ QWQLKYPKLI LREASSVSEE
 61 LHKEVQEAFL TLHKHGCLFR DLVRIQGKDL LTPVSPILIG NPGCTYKYLN TRLFTVPWPV
121 KGSNIKHTEA EIAAACETFL KLNDYLQIET IQALEELAAK EKANEDAVPL CMSADFPRVG
181 MGSSYNGQDE VDIKSRAAYN VTLLNFMDPQ KMPYLKEEPY FGMGKMAVSW HHDENLVDRS
241 AVAVYSYSCE GPEEESEDDS HLEGRDPDIW HVGFKISWDI ETPGLAIPLH QGDCYFMLDD
301 LNATHQHCVL AGSQPRFSST HRVAECSTGT LDYILQRCQL ALQNVCDDVD NDDVSLKSFE
361 PAVLKQGEEI HNEVEFEWLR QFWFQGNRYR KCTDWWCQPM AQLEALWKKM EGVTNAVLHE
421 VKREGLPVEQ RNEILTAILA SLTARQNLRR EWHARCQSRI ARTLPADQKP ECRPYWEKDD
481 ASMPLPFDLT DIVSELRGQL LEAKP
```

**FIGURE:** Mass analysis (LC/MS-MS) of native FTO.

# Products

## Antibodies

### anti-FTO (human), pAb

AG-25A-0084-C100 100 µg

From rabbit. **IMMUNOGEN:** Recombinant human FTO. **SPECIFICITY:** Recognizes human FTO. **APPLICATION:** WB.

### anti-FTO (mouse), pAb

AG-25A-0089-C100 100 µg

From rabbit. **IMMUNOGEN:** Recombinant mouse FTO. **SPECIFICITY:** Recognizes mouse FTO. Weakly cross-reacts with human FTO. **APPLICATION:** WB.

### anti-FTO (human), mAb (FT86-4)

AG-20A-0064-C050 50 µg
AG-20A-0064-C100 100 µg

**CLONE:** FT86-4. **ISOTYPE:** Mouse IgG1κ. **IMMUNOGEN:** Recombinant human FTO. **SPECIFICITY:** Recognizes human FTO. **APPLICATION:** IP, WB.

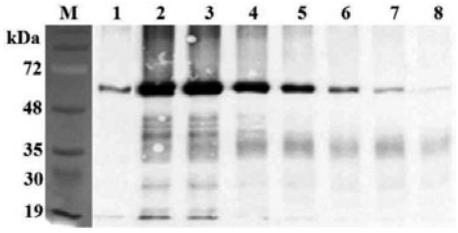


**FIGURE:** Immunoprecipitation of recombinant human FTO protein by anti-FTO (human), mAb (FT86-4). Recombinant human FTO protein at different concentrations was precipitated by anti-hFTO mAb (FT86-4). The precipitated protein was separated by SDS-PAGE, electroblotted, and visualized by Western blot with rabbit anti-mouse FTO pAb (AG-25A-0089).

1. rhFTO-His control 100ng (AG-40A-0112)
2. rhFTO-His 5µg
3. rhFTO-His 2.5µg
4. rhFTO-His 1.25µg
5. rhFTO-His 0.625µg
6. rhFTO-His 0.312µg
7. rhFTO-His 0.156µg
8. rhFTO-His 0.078µg

### new anti-FTO (mouse), mAb (FT342-1)

AG-20A-0088-C050 50 µg
AG-20A-0088-C100 100 µg

**CLONE:** FT342-1. **ISOTYPE:** Rat IgG2aκ. **IMMUNOGEN:** Recombinant mouse FTO. **SPECIFICITY:** Recognizes mouse FTO. Cross-reacts with rat FTO. Does not cross-react with human FTO. **APPLICATION:** WB.

### new anti-FTO (mouse), mAb (FT62-6)

AG-20A-0083-C050 50 µg
AG-20A-0083-C100 100 µg

**CLONE:** FT62-6. **ISOTYPE:** Mouse IgG1κ. **IMMUNOGEN:** Recombinant human FTO. **SPECIFICITY:** Recognizes mouse FTO. Weakly cross-reacts with human FTO. Does not cross-react with rat FTO. **APPLICATION:** IHC (PS), IP, WB.

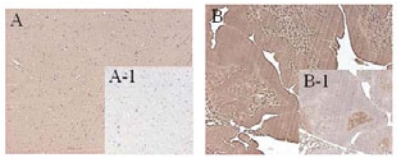


**FIGURE:** Immunohistochemical staining of FTO with anti-FTO (mouse) mAb (FT62-6) in mouse tissue (1:500 dilution, 200X).

A. Immunoperoxidase staining of formalin-fixed, paraffin-embedded mouse brain.
A-1. Isotype control, mouse brain.
B. Immunoperoxidase staining of formalin-fixed, paraffin-embedded mouse uterus.
B-1. Isotype control, mouse uterus.

## Proteins

### FTO (human) (rec.) (His)

AG-40A-0112-C010 10 µg
AG-40A-0112-C050 50 µg

Expressed in *E. coli*. The mature peptide of human FTO (aa 2-505) is fused at the N-terminus to a His-tag. **PURITY:** ≥90% (SDS-PAGE). **ENDOTOXIN CONTENT:** <1EU/µg protein (LAL-test).

### FTO (mouse) (rec.) (His)

AG-40A-0127-C010 10 µg
AG-40A-0127-C050 50 µg

Expressed in *E. coli*. The mature peptide of mouse FTO (aa 2-502) is fused at the N-terminus to a His-tag. **PURITY:** ≥90% (SDS-PAGE). **ENDOTOXIN CONTENT:** <1EU/µg protein (LAL-test).

### new FTO (rat) (rec.) (His)

AG-40A-0146-C010 10 µg
AG-40A-0146-C050 50 µg

Expressed in *E. coli*. The mature peptide of rat FTO (aa 2-502) is fused at the N-terminus to a His-tag. **PURITY:** ≥90% (SDS-PAGE). **ENDOTOXIN CONTENT:** <1EU/µg protein (LAL-test).

Purified (PF) = Purified (Preservative free); FACS = Flow Cytometry; ICC = Immunocytochemistry; IP = Immunoprecipitation; IHC = Immunohistochemistry (FS = Frozen Sections, PS = Paraffin Sections); WB = Western blot; BP = Blocking Peptide


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