

Supplementary Information to

***In vivo* analysis of internal ribosome entry at the *Hairless* locus by genome engineering**

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Supplementary Figure S1: The start sites in *Hairless*

Supplementary Figure S2: Survival rate of hemizygotes

Supplementary Figure S3: Raw data: Westernblot from Figure 5

Supplementary Figure S1

The start sites in *Hairless*

KpnI

ggtaccgtgccccaaaacatgaaaatggtgctagacgctcctaacgccgtgccgagctgg
catatTTTgcttagcgacgccgtcggctccgtTTTTcccgccggaactTTgcgcgagg

M1V AAGTG Intron 0
aatTTacaacaaaaacaatgaattaaATGgtattTTaaattgtccatataaatgcggtTTgg
M₁

Intron 0 GCCCTG
aaatgcgaaattgtgcctaaacttgcccaatgcaaacgaatgTTTctctTTgaagGCCCTG
A L 3

CTTAATGACG M1V M2V CAACAATGACCGATGAGCAT
CTTAATGACGTCACAAGCGTAGCAGAGTGCAACAGACAGACAACAATGACCGATGAGCAT
L N D V T S V A E C N R Q T T M₁₈ T D E H 23

AAAA M2V
AAAAGTAACATTAACAGTAACAGCAGTCACTCCAGCAACAACAACAACGGCAGCAGC
K S N I N S N S S H S S N N N N N G S S 43

AGCAATAACGACAACAACAGCAACGACGACGACGCAAGTAGCAGCAACAGCAAAAAACAAC
S N N D N N S N D D A A S S S N S K N N 63

AACACCAGCAACGAGAGCAGCCACAGCAACAACAATACTAGTAGCATAATTGCAGAGGCG
N T S N E S S H S N N N T S S I I A E A 83

GCCGCCAAGTTTCTACTGAAAAATGGCCTAAACGGCAGTAGCAGCACCAGCTACCCCCCT
A A K F L L K N G L N G S S S T S Y P P 103

AflIII
CTGCCACCGCCTCTGCCCCGCAACTTAAGCAGGACGACCACGCCACGACAACGACAACG
L P P P L P A N L S R T T T P T T T T T 123
K G R P R P R Q R Q R

CCCTCATCCTCCAGCTCCACCGCCTCAAAATGGCTTTTGGCGCATGCCAAGACGCCCAAA
P S S S S S T A S N G F L P H A K T P K 143
P H P P A P P P Q M A F C R M P R R P K

M3V TAGCATTGTTGGCTGCGTCCG M3V
AGTAGTAGCATTATGGCTGCGTCCGCCGAGTGGCAGCCAGCGTCGTTGGAGCTACTGCG
S S S I M₁₄₈ A A S A A V A A S V V G A T A 163
V V A L W L R P P Q W Q P A S L E L L R

TCCAAGCCCACCATCGATGTCCTGGGGGGCGTCTGGACTACAGTTCTTTGGGCGGAGCT
S K P T I D V L G G V L D Y S S L G G A 183
P S P P S M S W G A S Q T T V P W A E L

GCAACAGGCTCACTGCCCACCACTGCAGTAGTAGCGGCGGCAGCGGGAACAGCGAAGATC
A T G S L P T T A V V A A A A G T A K I 203
Q Q A H C P P L Q * * Cfs-construct

GGCAAGGGAAGCAACTCCGGCGGAAGCTTTGATATGGGCAGGACACCAATATCGACGCAC
G K G S N S G G S F D M G R T P I S T H 223
Intron 1

NT-Box
GGCAACAACAGCTGGGGCGGCTACGGTGGTCTGTTTGCAGTTCTTTAAAGATGGCAAATTC
G N N S W G G Y G G R L Q F F K D G K F 243

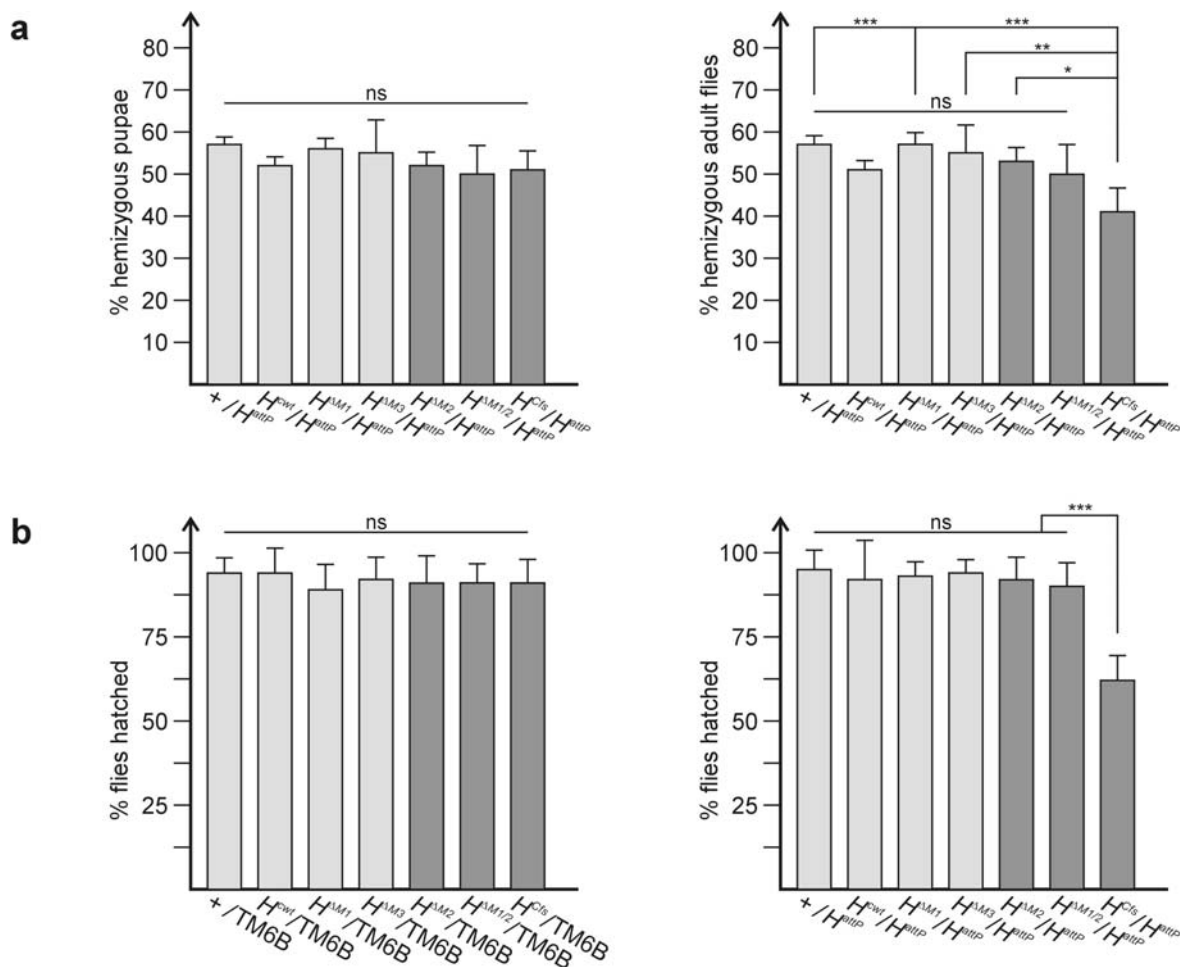
NT-Box
ATATTGGAAGTGGCGGGTCCAAGGATGGCGATAAAAGCGGCTGGGTTTCGGTCACG...
I L E L A R S K D G D K S G W V S V T . 263

Supplementary Figure S1

DNA and protein sequence of the Hairless 5' region is shown. The *Kpn* I site denotes the 5' end of all the constructs. Protein sequence is numbered, starting with Met₁. The NT-Box which mediates protein contacts to Su(H) is highlighted in orange; conserved residues are shown in bold, the ones directly contacting Su(H) are shown in red. The encoding sequence is split by Intron 1, indicated by a small arrow.

The three H start codons M1 (Met₁), M2 (Met₁₈) and M3 (Met₁₄₈) are highlighted in bold blue. M1 can only be used if Intron 0 (light grey) is spliced; the splice donor and acceptor as well as Stop codons within the intron are bold. M3 is used by internal ribosome entry; the presumptive IRES sequences are boxed.

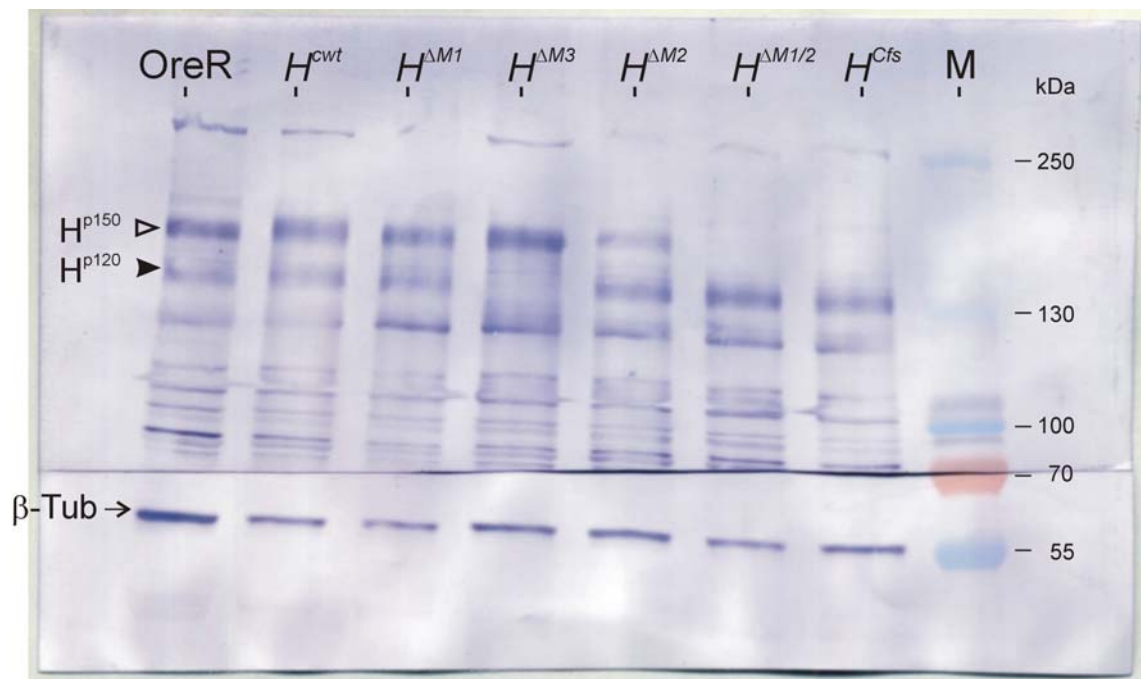
Primers M1V, M2V and M3V (turquoise) were used for *in vitro* mutagenesis of the Start codons to be replaced by Valine codons (GTG, bold purple). Note that mutagenesis was performed on cDNA not containing Intron 0. In the Cfs frame shift construct, a seven base pair deletion around the *Afl* II restriction site (bold, underlined) resulted in a frame shift at position 111 as depicted (grey, italics), ending translation prematurely at the novel codon 190. Hence, translation of H^{p120} can occur only by use of the IRES.



Survival rate of hemizygotes was determined by crossing the given homozygous H^* allele to the heterozygous H null allele $H^{attP} / TM6B$. Oregon 1 served as wild type control (+). Survival rate of hemizygous animals, i.e. the given allele *in trans* over the H^{attP} null allele, was assessed relative to balanced TM6B siblings.

(a) Left panel shows percentage of hemizygous pupae relative to balanced pupae: the expected 1:1 ratio is seen with all genotypes. Differences between genotypes are not significant (by ANOVA/Tukey-Kramer). Right panel compares numbers of hemizygous adults with their balanced siblings: here H^{Cfs} shows clear deficits.

(b) Shown is the percentage of adults that hatched from the pupal cases: whereas the balanced siblings (i.e. the heterozygotes) show no differences (left panel), H^{Cfs} flies appear in significantly lower numbers, whereas all other genotypes match the control (right panel).



Western blot of protein extracts from 3 mg of embryos per sample of the given genotype was probed for H protein expression. A step gel was used consisting 12% (lower 2/5th) and 7.5% (upper 3/5th) polyacrylamide. The two H protein isoforms H^{p150} (open arrowhead) and H^{p120} (closed arrowhead) are indicated. Beta-Tubulin (β -Tub) was used as loading control (arrow) on the same blot that was cut apart. Approximate size of protein standard (M) is given in kilo Dalton (kDa).