Inventory of supplemental information

Table S1

Amino acid sequences of all constructs. Related to Figures 2, 3, and 5.

Table S2

I_{50} values calculated from the plots shown in Figures 2, S2, and 3.

Figure S1

SDS-PAGE analysis of constructs with 1-5 glycosylated acceptor sites. Related to Figure 1.

Figure S2

Analysis of Lep^{IV} constructs with a 3K/7L/3G H3 segment lacking glycan acceptor site G3. Related to Figure 2.

Figure S3

SDS-PAGE analysis of truncated Lep^{V} constructs. Related to Figure 3.

Supplemental Table S1

In the Lep^V and Lep^{IV} sequences TMH1, TMH2 (in Lep^V), and the H1-H3 segment
underlined and the acceptor sites for N-linked glycosylation
are in bold. Positively charged Lys residues flanking H3 were introduced b
the Gly residues in the GGPG/GPGG flanks, and in cases with 5 flanking ch
also replacing the two amino acids adjacent to the tetrapeptide flank.
Sequences corresponding to gene fragments #1 and #2 (see Experimental Proc
are delineated by brackets in the Lep^V and Lep^{IV} sequences.

Amino acid sequence of Lep^V   363 amino acids

MAN<u>MFALILVIATLVTGILWCV</u>DKFFFAPKRRERQAAAQAAAGDSLDKATLKKVAPKPG<u>WLET
GASVFPVLAIVLIV</u>RSFIYEPFQIPG[GSMMPTL**NST**DFILVEAFAYGIADPIYQATLIETGH
PAPGEVGGPG<u>ALAALALALAALAALALAALA</u>GPGGLEYIAPAVGLPGD**NVT**YDPVSAELTIQPGC
SSGQACENGPGGPG<u>AAAALAAAALAAAALAAAA</u>GPGGQLSDFVQTFSPANGG**NAT**SGFFEVPA
QETAENGIALSETSGGPG<u>ALAALALAALAALALAALA</u>GPGGVPGQQQATWIVPAGQ**NAT**MGDN
AD**NST**DSAYWGFVPEANLVGRATAIWMSFDKQEGEWPTGLRLSRIGGIH**PSSFTLSPEL]

Amino acid sequence of Lep^{IV}   386 amino acids

MA**NST**SQGSQPINAQAAPVAQGGSQGE<u>FALILVIATLVTGILWCV</u>DKFFFAPKRRERQAAAQAA
AGDSLDKATLKKVAPK[TSGGPG<u>ALAALALAALAALALAALA</u>GPGGVPIPSGSMMPTL**NST**DFI
LVEAFAYGIADPIYQATLIETGHPAPGEVGGPG<u>AAAALAAAALAAAALAAAA</u>GPGGLEYIAPAV
GLPGD**NVT**YDPVSAELTIQPGCSSGQACENGPGGPG<u>ALAALALAALAALALAALA</u>GPGGQLSDF
VQTFSPANGG**NAT**SGFFEVPAQETAENGIRLSERKETLGDVTHRILTVPIAQDQVGMYYQQPGQ
QLATWIVPAGQ**NAT**MGDNRDNSADSRYWGFVPEANLVGRATAIWMSFDKQEGEWPTGLRLSRIG
GIH**PSSFTLSPEL]

Constructs based on Lep^V

ID	composition of H2	composition of H3	series
1	GGPGAAAAAAAAAAAAAAAAAAAGPGG	GGPGALAALALAALAALALAALAGPGG	1
2	GGPGAAAAAAAAALAAAAAAAAAGPGG	GGPGALAALALAALAALALAALAGPGG	1
3	GGPGAAAALAAAAAAAAALAAAAGPGG	GGPGALAALALAALAALALAALAGPGG	1
4	GGPGAAAALAAAALAAAALAAAAGPGG	GGPGALAALALAALAALALAALAGPGG	1
5	GGPGAAAALALAAAAALALAAAAGPGG	GGPGALAALALAALAALALAALAGPGG	1
6	GGPGAAAALALAALAALALAAAAGPGG	GGPGALAALALAALAALALAALAGPGG	1
7	GGPGAAAALALALALALALAAAAGPGG	GGPGALAALALAALAALALAALAGPGG	1
8	GGPGALAALALAALAALALAALAGPGG	GGPGALAALALAALAALALAALAGPGG	1
9	GGPGAAAAAAAAAAAAAAAAAAAGPGG	KKPKALAALALAALAALALAALAGPGG	2
10	GGPGAAAAAAAAALAAAAAAAAAGPGG	KKPKALAALALAALAALALAALAGPGG	2
11	GGPGAAAALAAAAAAAAALAAAAGPGG	KKPKALAALALAALAALALAALAGPGG	2
12	GGPGAAAALAAAALAAAALAAAAGPGG	KKPKALAALALAALAALALAALAGPGG	2
13	GGPGAAAALALAAAAALALAAAAGPGG	KKPKALAALALAALAALALAALAGPGG	2

Supplemental Table S2

I_{50} values calculated from the plots shown in Figures 2, S2, and 3.

Figure 2   Lep^{IV}

I_{50}	7L	9L	11L	19L
5K/nL/3G	3			2.6
3K/nL/3G	3.6			
3G/nL/3G	3.5			4.6
3G/nL/3K	4.8	4.5	4.4	
3G/nL/5K	> 7			> 7

Figure S2

3K/nL/3G	3.6			

Figure 3   Lep^{V}

I_{50}	0L	7L	9L	11L	19L
5K/nL/3G		3.5			3.4 (3.0*)
3K/nL/3G		3.3			
3G/nL/3G	2.9	2.8		2.2	
3G/nL/3K		1.2	1.3	0.6	< 0
3G/nL/5K		0.7			< 0

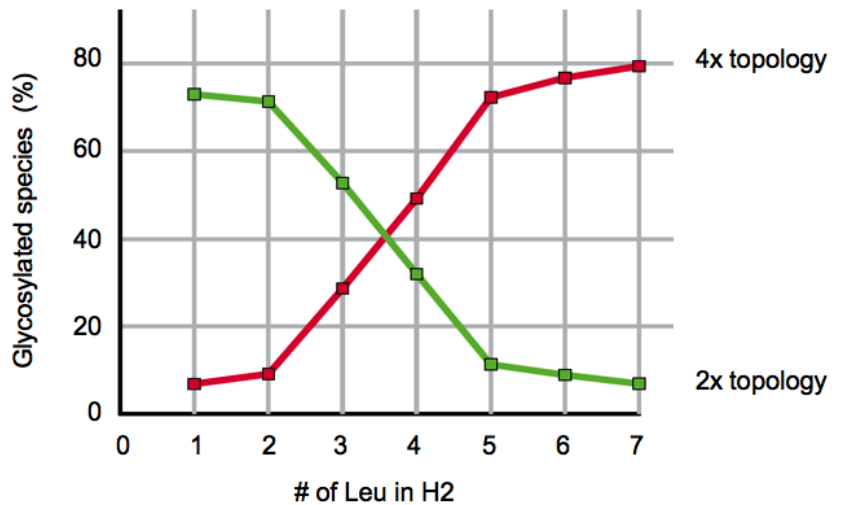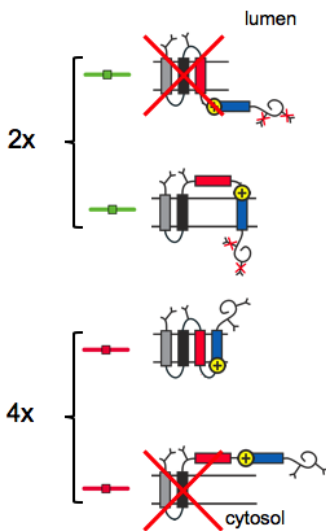*lower Mw form not included in the quantitation, see main text.

Figure S1, related to Figure 1
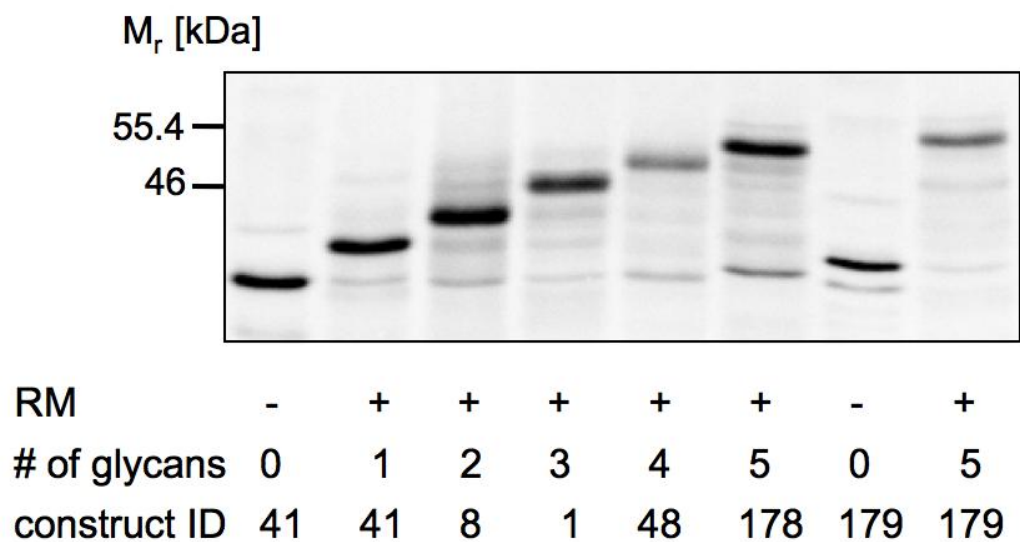
Expression of different constructs (see ID numbers in Table S1 for sequences) glycosylated on zero to five engineered glycosylation acceptor sites. RM = rough microsomes.

Figure S2, related to Figure 2

Membrane insertion of Lep^{IV} constructs lacking the G3 glycosylation site between H2 and H3 into dog pancreas rough microsomes. The H2 test segments are of varying hydrophobicity (n_{H2} = 1-7). H3 has the composition 3K/7L/3G. The graph shows the fractions of the two dominating species with, respectively, two (green) and four (red) glycans. The different topologies consistent with the observed number of glycans are indicated on the left; however, since the H3 segment has a high hydrophobicity, the crossed-out topologies can be ruled out.

Figure S3, related to Figure 3

In vitro translation of Lep^V constructs in the absence (-) and presence (+) of rough microsomes (RM). Digestion of N-linked glycans by endoglycosidase H (EndoH) was carried out on the sample in lane 3. All constructs have a 3G/0L/3G H2 segment and a 5K/19L/3G H3 segment (c.f., Fig. 3). Lanes 1-3: full-length construct. Lanes 4-5: construct with tandem stop codons (*) placed immediately after H2 (...AAAG**). Lanes 6-7: construct with tandem stop codons (*) placed immediatley after H3 (...LLLG**). Lanes 8-9: construct starting at Met^{92} in Lep. White circles indicate unglycosylated full-length protein; black circles indicate full-length protein glycosylated on one, two, and three acceptor sites; white squares indicate unglycosylated truncated protein; and black squares indicate glycosylated truncated protein.