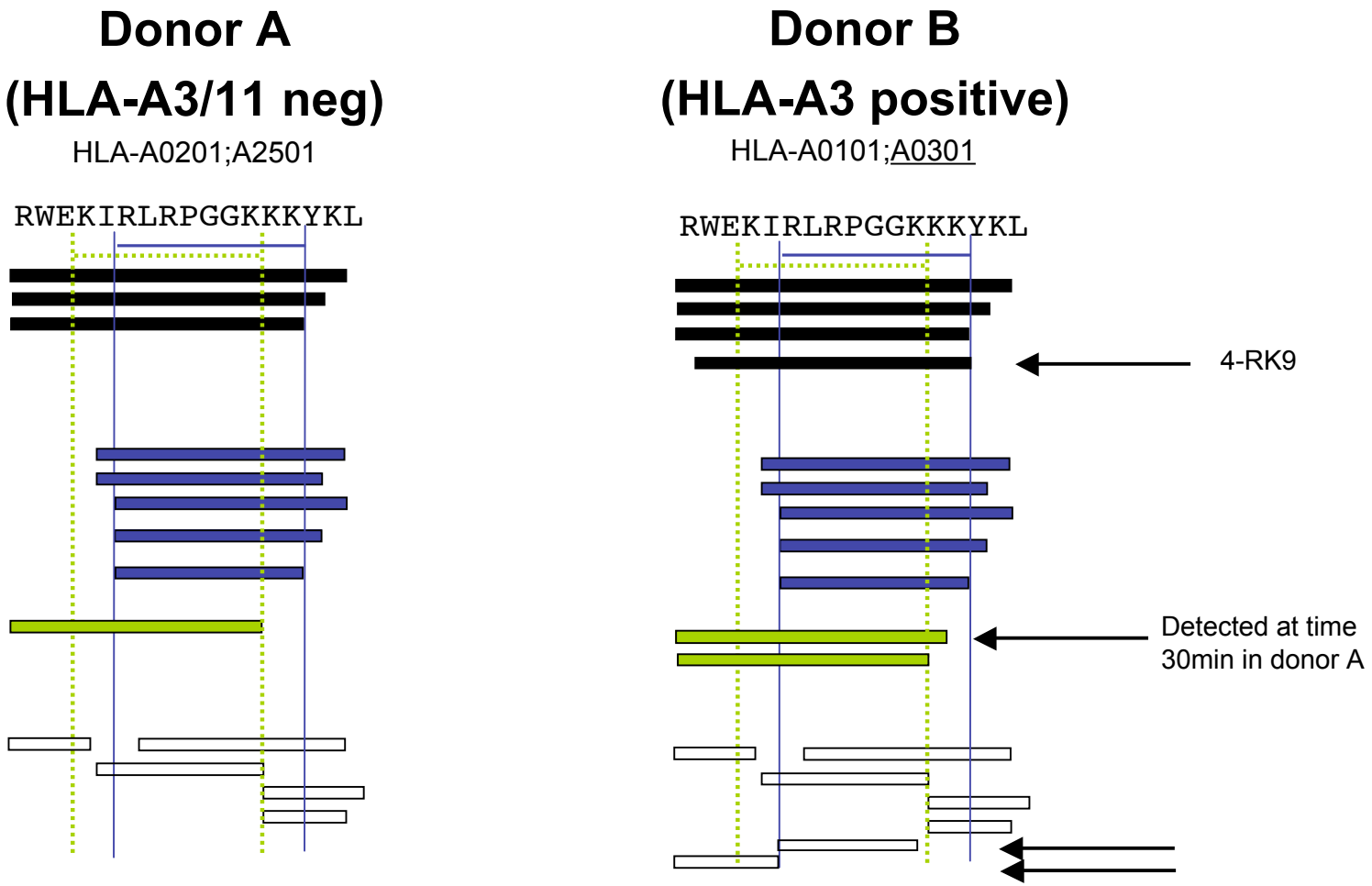
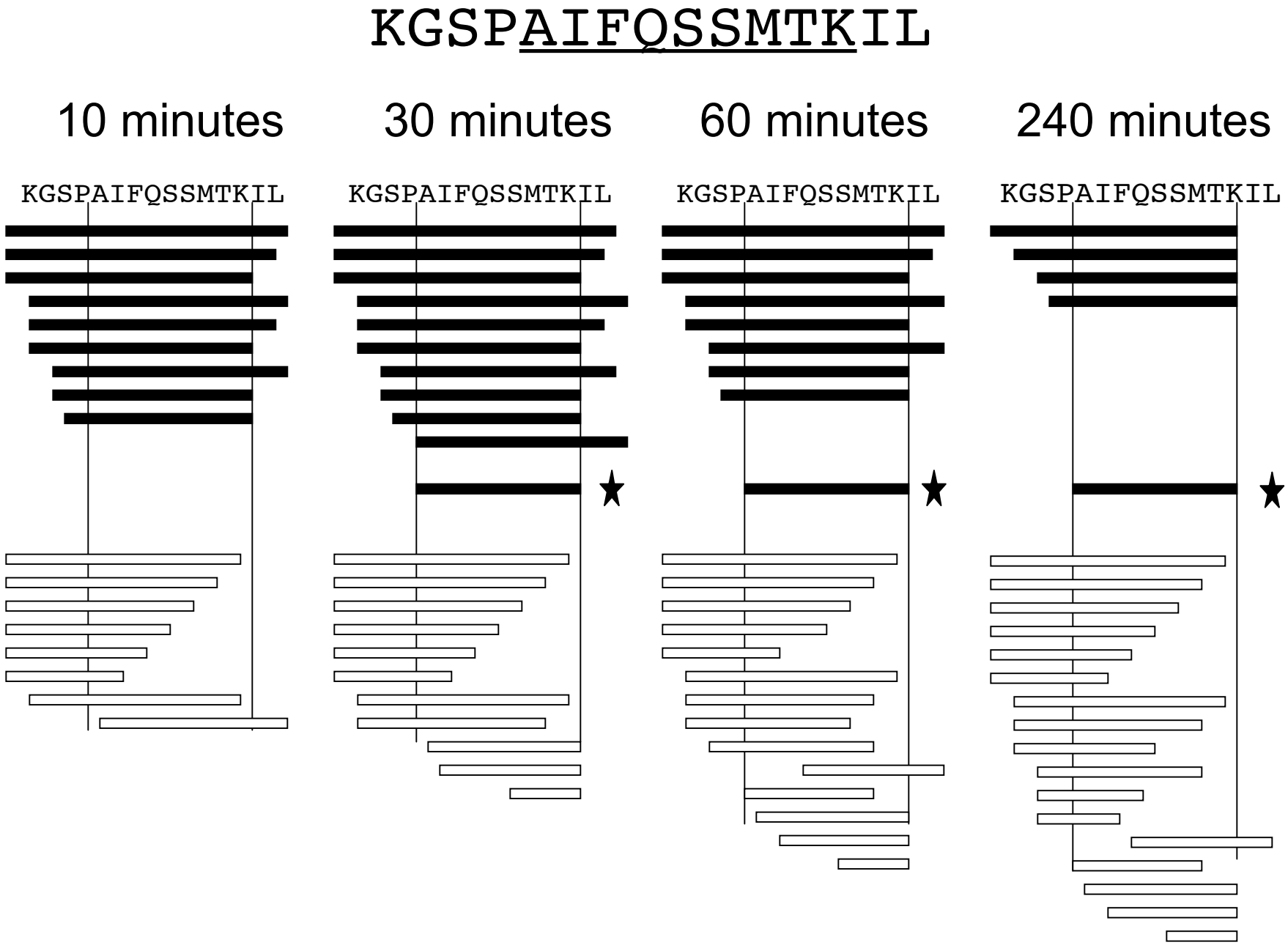
Supplementary figure 1

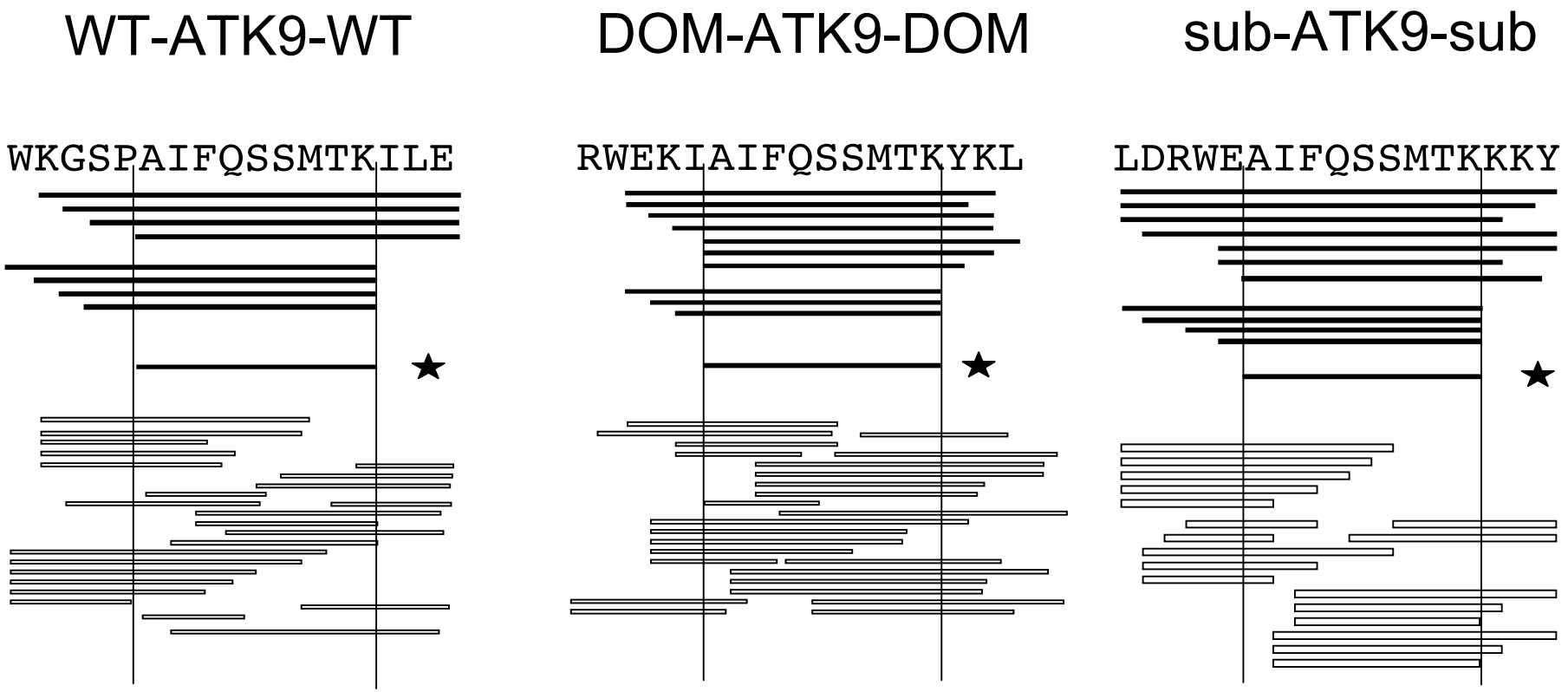
RWEKIRLRPGGKKKYKL



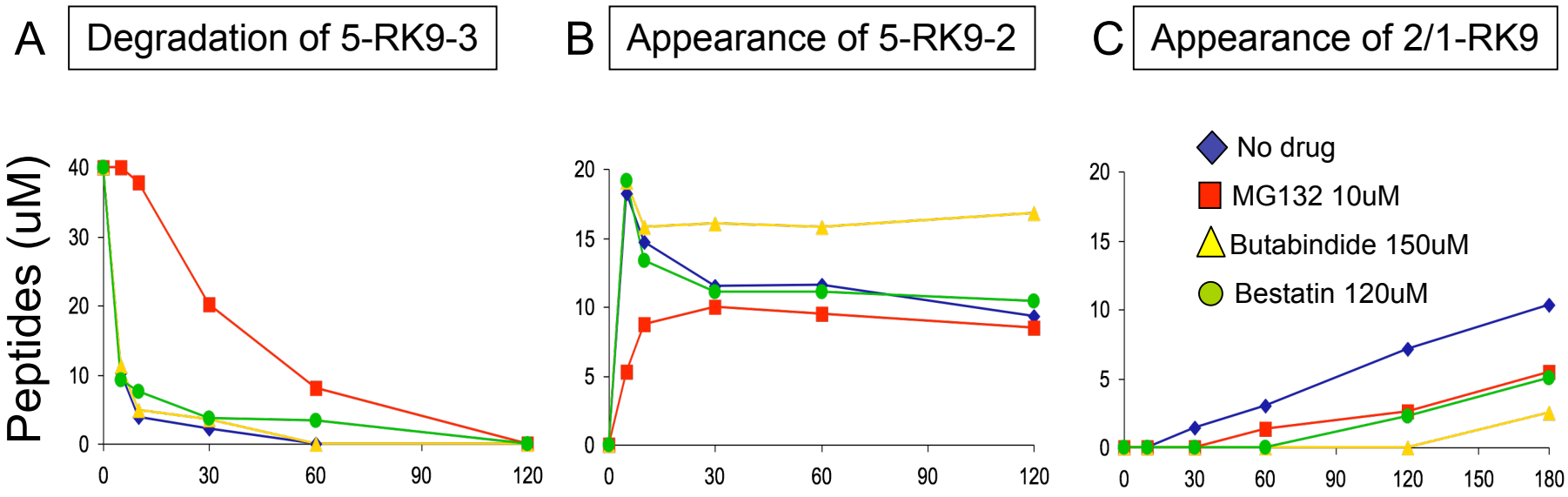
Supplementary figure 2



Supplementary figure 3

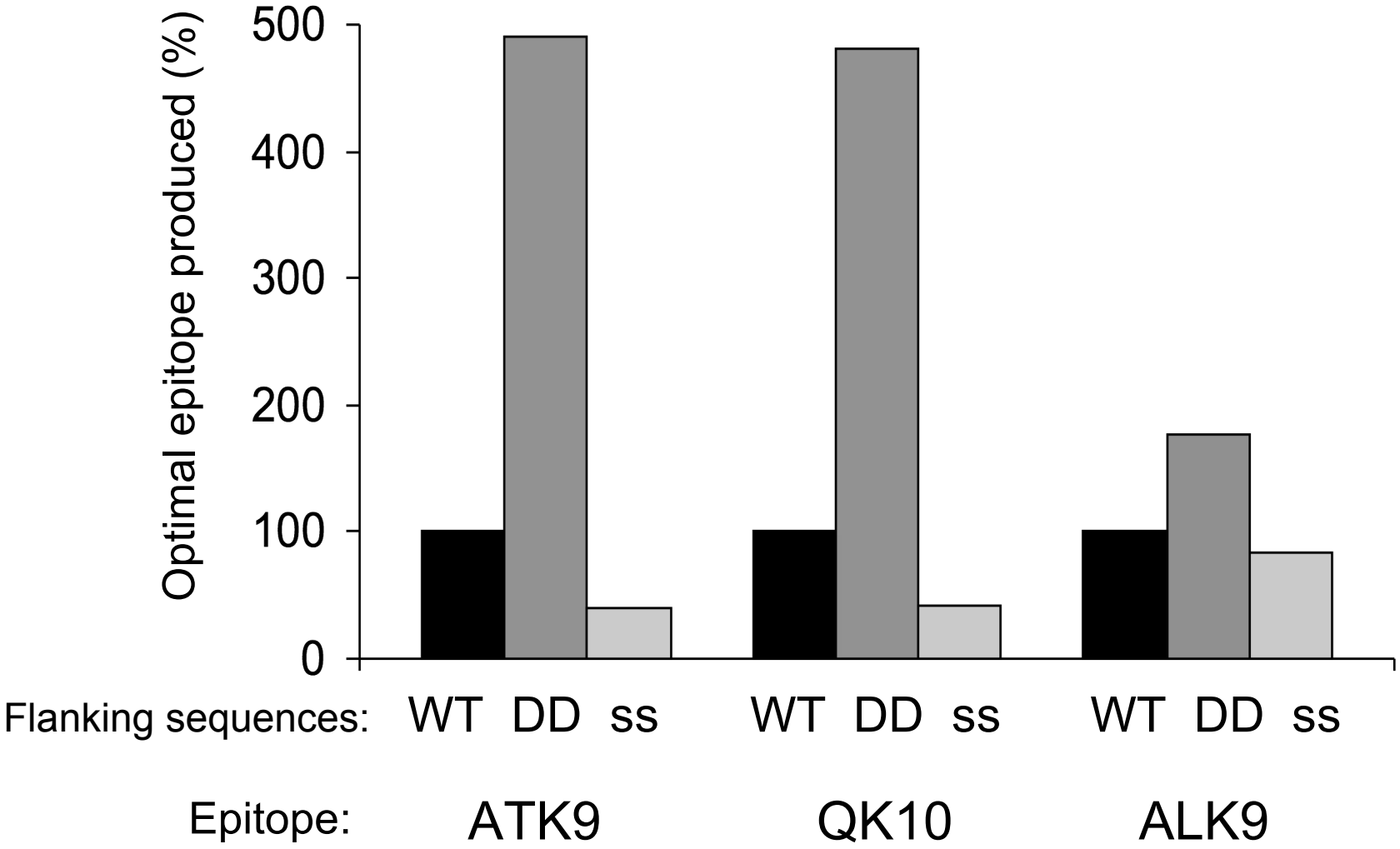


Supplementary figure 4



5-RK9-3 —Proteasome→ 5-RK9-2 —TPPII→ —aminopeptidases→ —→ —→ 2/1-RK9

Supplementary figure 5



Supplementary figure 1: The cytosolic degradation of Gag 17-mer leads to the production of mostly identical peptides independent of the HLA type of the donor.
8nmol of a Gag 17-mer were incubated with 40ug PBMC cytosol from Donor A (HLA-A03/11 negative) or Donor B (HLA-A0301 positive) for 1 hour. Peptides encompassing both epitopes (black bars), RK9 only (blue bars), KK9 only (green bars) or no epitope (white bars) were identified by mass spectrometry. Degradation products differing between donor A and B are indicated with an arrow. The degradation of 4-RK9 yielded 2/1-RK9 and other shorter peptides with extracts from both donors (not shown).

Supplementary figure 2: The cytosolic degradation of 4-ATK9-2 leads to the production of optimal ATK9 epitope.
8nmol of 4-ATK9-2 were incubated with 40ug PBMC cytosol for increasing periods of time. Peptides encompassing ATK9 (black bars) or no epitope (white bars) were identified by mass spectrometry. Optimal ATK9 is indicated with a black star. The data are representative of 3 experiments performed with extracts from 3 healthy donors.

Supplementary figure 3: The degradation of 5-ATK9-3 and hybrid ATK9 with Gag flanking sequences lead to the production of optimal ATK9 epitope.
8nmol of WT-ATK9-WT, DOM-ATK9-DOM or sub-ATK9-sub (left through right) were incubated with 40ug PBMC whole cell extracts for increasing periods of time. Peptides encompassing ATK9 (black bars) or no epitope (white bars) were identified by mass spectrometry. Optimal ATK9 is indicated with a black star. The data are representative of 3 experiments performed with extracts from 3 healthy donors.

Supplementary figure 4: The degradation of Gag 17-mer into short peptides requires proteasome, TPPII and aminopeptidases. PBMC extracts were preincubated for 30 minutes without drug (blue diamond), MG132 10uM (red square), butabindide 150uM (yellow triangle), bestatin 120uM (green circle) before addition of Gag 17-mer. The identity and amount of various peptides was analyzed by HPLC (A-C) and confirmed by mass spectrometry. (A) Degradation of Gag 17-mer (5-RK9-3). (B) Appearance of 5-RK9-2. (C) Appearance of 2/1RK9. Data are representative of 3 experiments performed with extracts from 3 healthy donors.

Supplementary figure 5: Portable flanking sequences alter the production of 3 epitopes. The in vitro degradation of several 17-mers into optimal epitopes was analyzed as described in supplementary figure 2. From left to right: HLA-A3/11 RT ATK9 with WT (black), DD (dark grey), ss (light grey) flanking sequences; HLA A3/11-restricted Nef QK10 (QVPLRPMTYK, residues 73-82) with WT (FPTVPQVPLRPMTYKAAV), DD or ss flanking sequences, HLA A3-restricted subdominant Nef ALK9 (AVDLSHFLK; residues 84-92) with WT (MTYKAAVDLSHFLKEKG), DD or ss flanking sequences. Optimal epitopes were identified and quantified by mass spectrometry and RP-HPLC. 100% is assigned to the amount of optimal epitope produced from the WT 17-mer.
The data are representative of 3 experiments performed with extracts from 3 healthy donors.