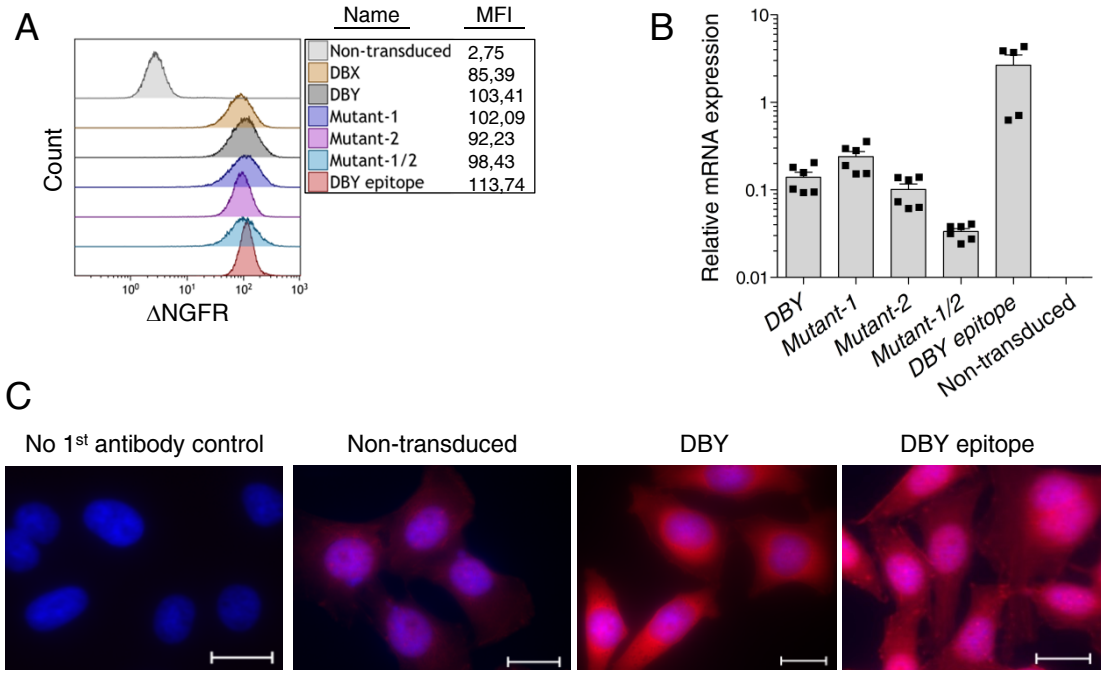
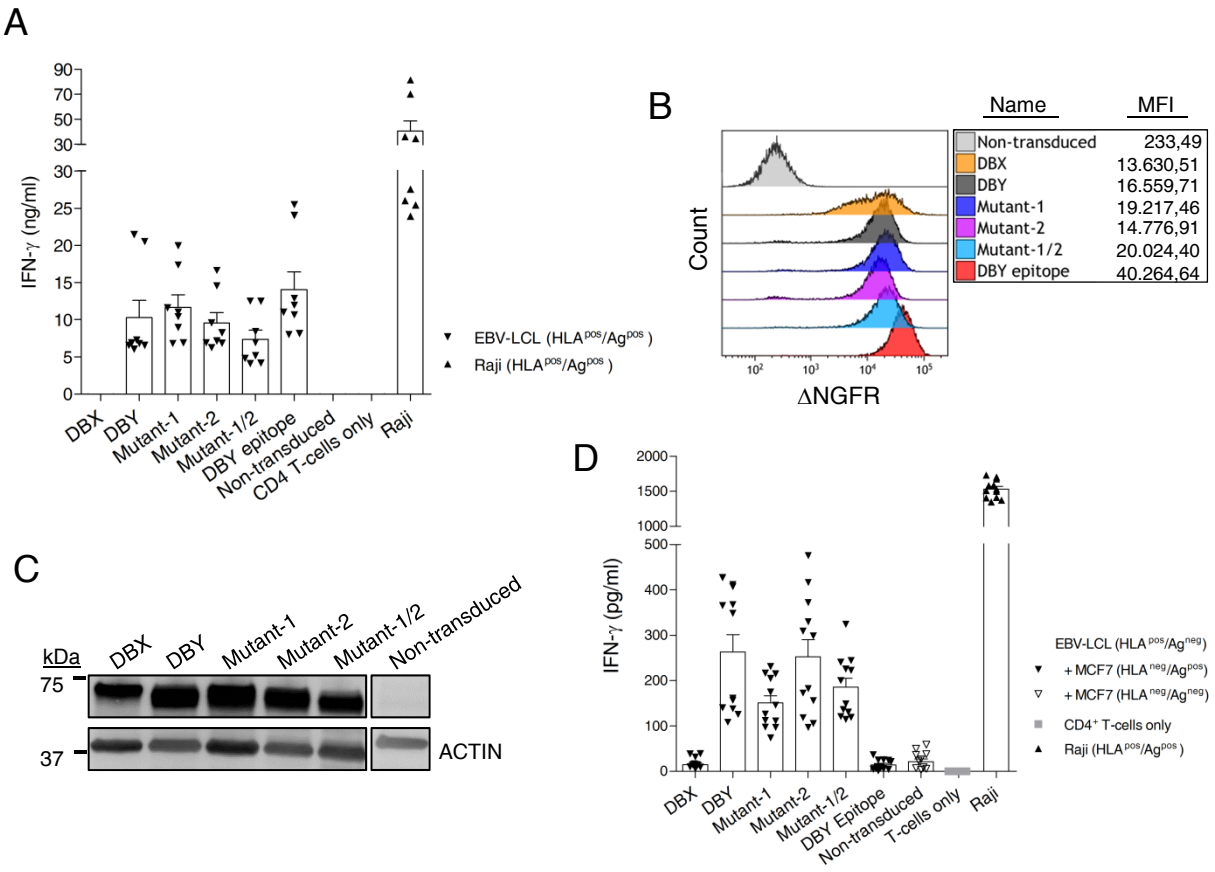
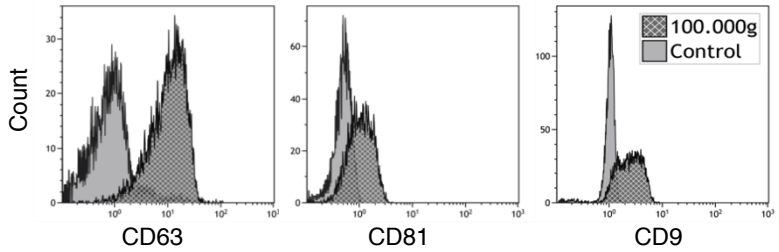


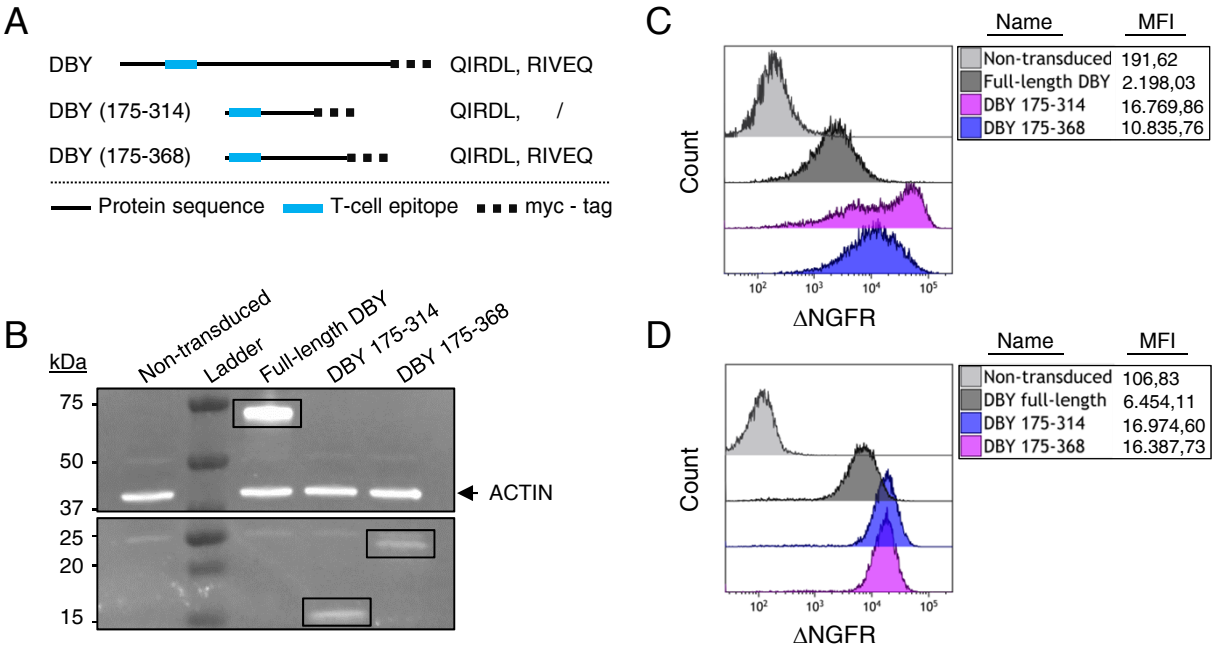
Supplemental Figure 1. Characterization of retrovirally transduced HeLa cells.
(**A**) After retroviral transduction and cell sorting of HeLa cells, marker gene expression (ΔNGFR) was analyzed on the flow cytometer. Values represent the mean fluorescence intensity (MFI) of the marker gene. (**B**) Relative mRNA expression is shown for transgene-positive HeLa cells. Data represent the mean ±SEM (*n* = 2). (**C**) Merged immunofluorescence images of transgene-positive HeLa cells (red) with DAPI nuclear stain (blue). X630. Scale bars: 20 µm.



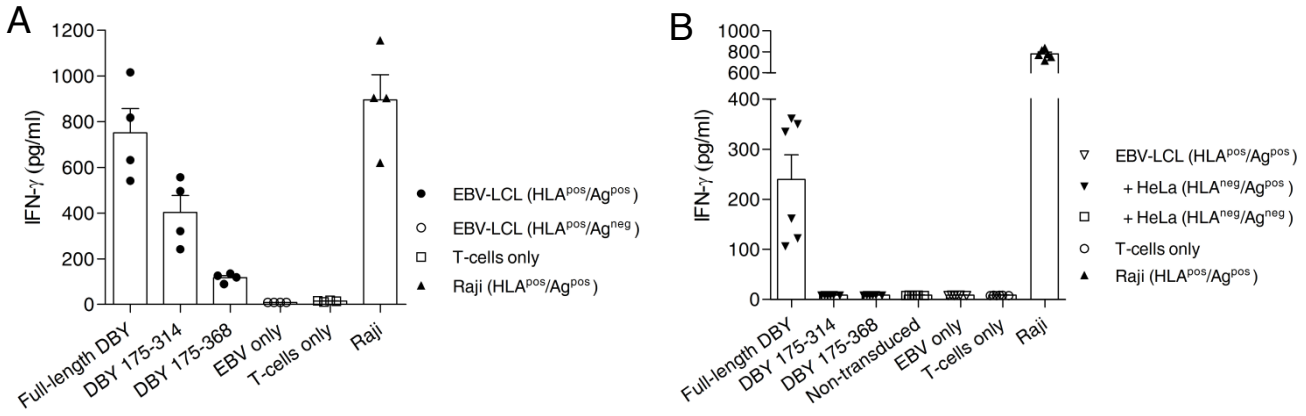
Supplemental Figure 2. Direct and indirect antigen presentation.
(**A**) EBV-LCL were retrovirally transduced with indicated DBY-constructs and cell sorted. HLA-II-positive and antigen-positive (HLA^{pos}/Ag^{pos}) EBV-LCL were co-cultured with a DBY-specific CD4+ T-cell clone to assess antigen processing and presentation by T-cell activation in IFN-γ ELISA. Data show the mean ±SEM ($n = 4$). (**B**) Marker gene expression (ΔNGFR) and mean fluorescence intensity (MFI) of retrovirally transduced and cell sorted MCF7 cell lines is illustrated. (**C**) Western blot analysis of retrovirally transduced MCF7 cell lines. (**D**) HLA-II-negative and antigen-positive MCF7 cells (HLA^{neg}/Ag^{pos}) were co-cultured with HLA-II-positive and antigen-negative EBV-LCL (HLA^{pos}/Ag^{neg}). After co-culture, the DBY-specific CD4+ T-cell clone was added and T-cell activation measured in IFN-γ ELISA. Data were normalized to marker gene expression. Shown is the mean ±SEM ($n = 2$) of six replicates.



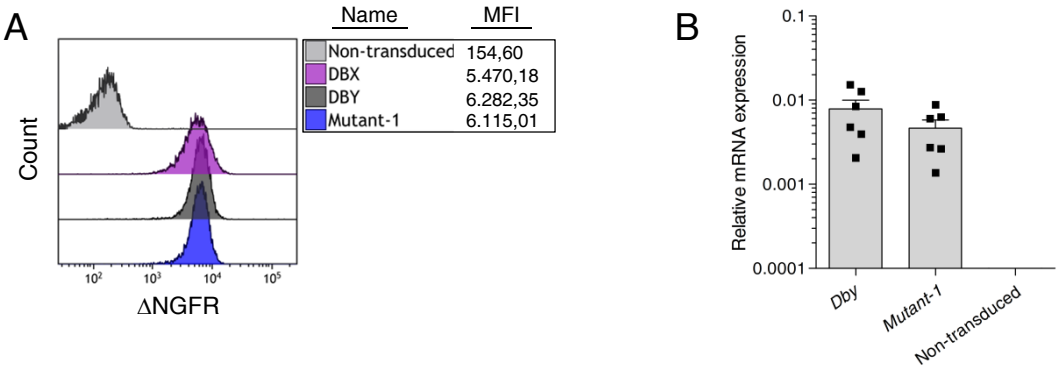
Supplemental Figure 3. Characterization of the ultracentrifuged fraction.
After ultracentrifugation, anti-CD63 magnetic beads were incubated with the pelleted fraction. Subsequently, beads were stained with fluorescently labeled antibodies against CD63, CD81 and CD9 and analyzed on the flow cytometer. As control, beads were stained solely with fluorescently labeled antibodies.



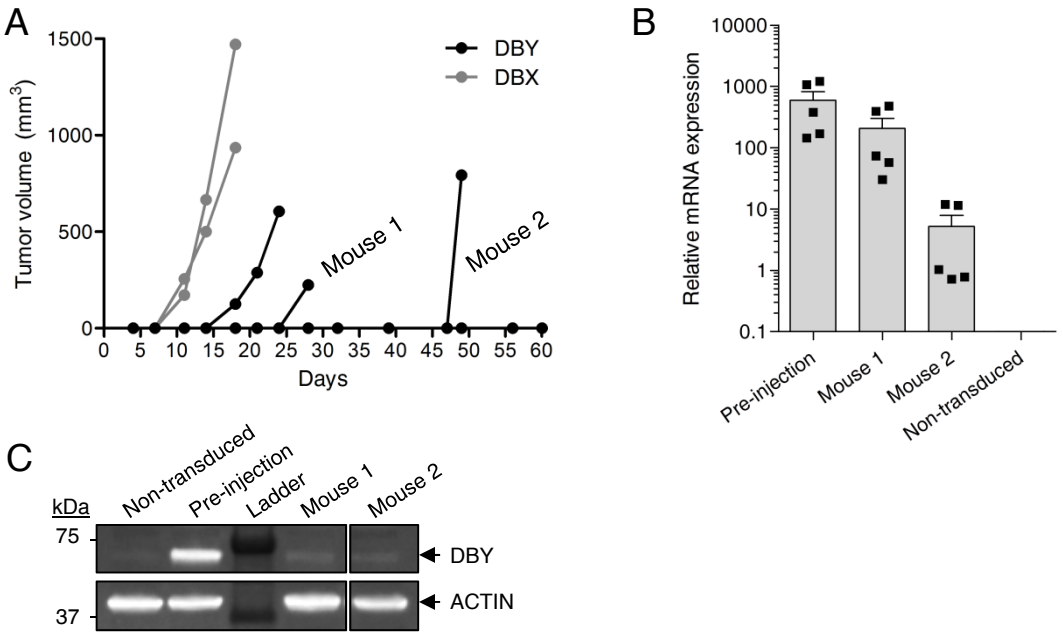
Supplemental Figure 4. Characterization of retrovirally transduced and truncated DBY protein-variants.
(**A**) Schematic representation of full-length wildtype DBY and truncated DBY proteins. QIRDL (motif-1), RIVEQ (motif-2). (**B**) Western blot analysis of HeLa cells expressing full-length DBY or truncated DBY proteins. Rectangles indicate full-length DBY (74.3 kDa), DBY 175-314 (16.9 kDa) or DBY 175-368 (23.2 kDa). (**C** and **D**) After retroviral transduction and cell sorting of EBV-LCL (**C**) or HeLa cells (**D**) marker gene expression (ΔNGFR) was analyzed by flow cytometry. Values represent the mean fluorescence intensity (MFI) of the marker gene.



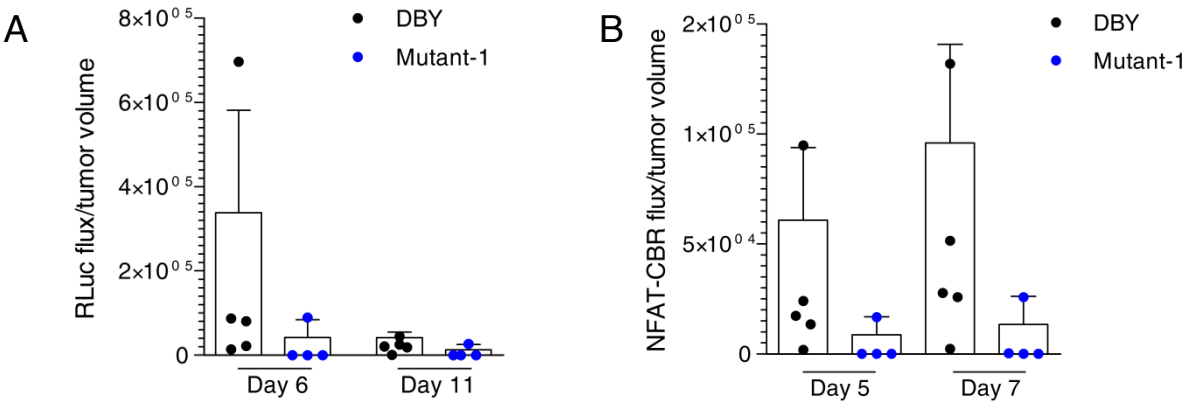
Supplemental Figure 5. Direct and indirect antigen presentation of truncated DBY-proteins. (**A**) Direct antigen presentation: HLA-II-positive and antigen-positive (HLA^{pos}/Ag^{pos}) EBV-LCL were co-cultured with a DBY-specific CD4+ T-cell clone to assess T-cell activation in IFN-γ ELISA. Data represent the mean ±SEM of duplicated wells (n = 2). (**B**) Indirect antigen presentation: HLA-II-negative and antigen-positive HeLa cells (HLA^{neg}/Ag^{pos}) were co-cultured with HLA-II-positive and antigen-negative (HLA^{pos}/Ag^{neg}) EBV-LCL. After co-incubation, EBV-LCL were isolated and tested for T-cell recognition in IFN-γ ELISA. Data are shown as the mean ±SEM of triplicated wells (n = 2).



Supplemental Figure 6. Transgene expression in retrovirally transduced EL-4 cell lines. (**A**) Marker gene expression (ΔNGFR) of the established EL-4 cell lines was analyzed by flow cytometry. Values represent the mean fluorescence intensity (MFI) of the marker gene. (**B**) Relative mRNA expression in EL-4 cells is depicted. Shown is the mean ±SEM of triplicated wells (n = 2).



Supplemental Figure 7. Ex-vivo analysis of full-length DBY expressing EL-4 tumors.
(**A**) Tumor growth in female Marilyn mice after challenge with 1x10^5 EL-4 tumor cells transduced with full-length DBX (n = 2) or DBY (n = 4). (**B**) Relative mRNA expression of murine full-length *Dby* in ex vivo analyzed EL-4 tumors. Shown is the mean ±SEM of at least duplicated wells (n = 2). (**C**) Western blot analysis of ex vivo analyzed EL-4 tumors.



Supplemental Figure 8. In vivo bioluminescent flux analysis.
Depicted is in vivo bioluminescent signal strength (p/sec/cm^2/sr) divided through the current tumor volume (mm^3) at indicated days in each albino B6 mice after challenge with EL-4 tumors and adoptive transfer with Marilyn-BLITC-derived antigen-specific CD4+ T-cells. (**A**) Antigen-specific T-cell migration/expansion (Rluc signal). (**B**) NFAT-activation in antigen-specific CD4+ T-cells (NFAT-CBR signal). Illustrated is the mean ±SEM (n = 4-5).

Supplemental Table 1. Oligonucleotides used to amplify full-length and truncated genes

Designation	Sequence (5' to 3')
Human *DBX*	F: CGCGGATCCGGGATGAGTCATGTGGCAGTGGA
	R: CCGGAATTCGTATCA**GAGATCCTCCTCTGAGAT GAGCTTTTGCTC**GTTGCCCCACCAGTCAACCC
Human *DBY*	F: CGCGGATCCGGGATGAGTCATGTGGTGGTGAA
	R: CCGGAATTCGTATCA**GAGATCCTCCTCTGAGAT GAGCTTTTGCTC**GTTGCCCCACCAGTCAACCC
Human *DBY*	F: CGCGGATCCGGGATGCCACATATTGAGAATTT
amino acids 175-314	R: CCGGAATTCGTATCA**GAGATCCTCCTCTGAGAT GAGCTTTTGCTC**TCCACGTTCTAAGTCCCGAA
Human *DBY*	F: CGCGGATCCGGGATGCCACATATTGAGAATTT
amino acids 175-368	R: CCGGAATTCGTATCA**GAGATCCTCCTCTGAGAT GAGCTTTTGCTC**CATAGTATCTTGTTCAACTA
Murine *Dbx*	F: CGCACGCGTGGGATGAGTCATGTGGCAGTGGA
	R: CCGGAATTCGTATCA**GAGATCCTCCTCTGAGAT GAGCTTTTGCTC**GTTACCCCACCAGTCAACCC
Murine *Dby*	F: CGCACGCGTGGGATGAGTCAAGTGGCAGCGGA
	R: CCGGAATTCGTATCA**GAGATCCTCCTCTGAGAT GAGCTTTTGCTC**ATTGCCCCACCAGTCAACTG

F: forward, R: reverse; Underlined nucleotides indicate restriction sites
Boldface nucleotides indicate the fused myc-tag at the C-terminus

Supplemental Table 2. Oligonucleotides designed to clone the CD4+ T-cell epitope of human DBY

Designation	Sequence (5' to 3')
Human *DBY* CD4+ T cell epitope	F: CGCGGATCCGGGATGCCACATATTGAGAATTTTA GCGATATTGACATGGGAGAAATT**GAGCAAAAGCT CATCTCAGAGGAGGATCTC**TGATACGAATTCCGG
	R: CCGGAATTCGTATCA**GAGATCCTCCTCTGAGATG AGCTTTTGCTC**AATTTCTCCCATGTCAATATCGCT AAAATTCTCAATATGTGGCATCCCGGATCCGCG

F: forward, R: reverse; Underlined nucleotides indicate restriction sites;
Boldface nucleotides indicate the fused myc-tag at the C-terminus

Supplemental Table 3. Oligonucleotides used for site-directed mutagenesis

Designation	Sequence (5' to 3')
Human *DBY* Motif: QIRDL to AIADL	F: TTATGGTGGTGCTGATATTGGTCAG**GC**GATT**GC** GGACTTAGAACGTGGATGCCACTTGTTAGTAG
	R: CTACTAACAAGTGGCATCCACGTTCTAAGTCC**GC** AATC**GC**CTGACCAATATCAGCACCACCATAA
Human *DBY* Motif: RIVEQ to RIVAA	F: TATGGGATTTGAACCTCAGATACGTCGTATAGT TG**C**A**GC**AGATACTATGCCACCAAAGGGCGTTC
	R: GAACGCCCTTTGGTGGCATAGTATCT**GC**T**G**CAA CTATACGACGTATCTGAGGTTCAAATCCCATA
Murine *Dby* Motif: QIRDL to AIADL	F: GTATGGTGGTGCTGATACTGTTCAG**GC**GATT**GC** GGACTTAGAACGTGGATGCCACTTGTTAGTTG
	R: CAACTAACAAGTGGCATCCACGTTCTAAGTCC **GC**AATC**GC**CTGAACAGTATCAGCACCACCATAC
Murine *Dby* Motif: RIVEQ to RIVAA	F: TATGGGATTTGAACCTCAAATACGTCGTATAGT TG**C**G**GC**GGACACAATGCCACCAAAGGGGGTTC
	R: GAACCCCCTTTGGTGGCATTGTGTCC**GC**C**G**CAA CTATACGACGTATTTGAGGTTCAAATCCCATA

F: forward, R: reverse; Boldface indicates mutated nucleotides

Supplemental Table 4. Oligonucleotides used for sequence analysis

Designation	Sequence (5' to 3')	Gene position
MP71.60 leader	F_1: GTCTCTGTCTGACTGTGTTTCTGTATT	Vector pre-MCS
Human *DBX*	F_2: AACAGGGTCTGGAAAAAC	675 - 692
	F_3: GGCTGTAGGAAGAGTTGG	1209 - 1226
Human *DBY*	F_2: AGGATCTGGGAAAACTGC	672 - 689
	F_3: GCAACAGGGAGTGATTCA	1303 - 1320
Murine *Dbx*	F_2: TCAAACAGGCTCTGGAAA	672 - 689
	F_3: GGCTGTAGGAAGAGTTGG	1209 - 1226
Murine *Dby*	F_2: AACAGGGTCTGGAAAAAC	672 - 689
	F_3: AAATGCAACAGGGAAGGA	1302 - 1319

F1-3: forward primer, MCS: Multiple-cloning-site

Supplemental Table 5. Oligonucleotides used for real-time PCR

Designation	Sequence (5' to 3')	Gene position
Human *DBY*	F:CGGTACACCACATATTGAGA	516 - 535
	R:TTCTCCCATGTCAATATCGC	561 - 542
Human *18S rRNA*	F:ACCGATTGGATGGTTTAGTGAG	1715 - 1736
	R:CCTACGGAAACCTTGTTACGAC	1847 - 1826
Murine *Dby*	F:AGCCGAAGTAGTGGTAGTAG	1855 - 1874
	R:ACCTCCACCAAATCCTCTGT	1899 - 1880
Murine *18S rRna*	F:CGCCGCTAGAGGTGAAAT	950 - 967
	R:CGAACCTCCGACTTTCGT	1033 - 1047

F: forward, R: reverse; Underlined nucleotides do not correspond to the original sequence