SUPPLEMENTAL FIGURES AND FIGURE LEGENDS



5' CTGGAAGAGCTGAAGTGC	CACATATGC	TCTGTTTAGGAGAGCAAGAACTACAAATT
3'	165.18 <mark>L</mark>	JAGACAAAUCCUCUCGUU
	190.18	UCUCGUUCUUGAUGUUUA
	190.15	UCGUUCUUGAUGUUU
	191.18	UUAA
TGGTCTTCAGTTTGGCTTGC	TTACATCCTG	AGAACTCTGTAGGCCACATGTCGTGAA
ACCAGAAGUCAAAC		
186.18	AUGUAGGAC	CUCUUGAGAC
186.15	UGUAGGAC	CUCUUGAG
186.12	UGUAGGAC	CUCUU
5' 186.12	UAGGAC	CUCUUG
186.25 ACC	BAAUGUAGGA	CUCUUGAGACAUC
		GAA.12 AGCACUU
	GTGAAAGCCAG	GAA.12 AGCACUU GAAAAGGAAGTGGTAAGTCTCAGGGGAG 3'
TATAGCAGCCTCTGCAACAC		GAA.12 AGCACUU GAAAAGGAAGTGGTAAGTCTCAGGGGAG 3' UUUCCUUCACCAUUCAGA
TATAGCAGCCTCTGCAACAC AUAUC	CTGAAAGCCA 217.18 187.18	GAA.12 AGCACUU GAAAAGGAAGTGGTAAGTCTCAGGGGAG 3' UUUCCUUCACCAUUCAGA UUCCUUCACCAUUCAGAG
TATAGCAGCCTCTGCAACAC AUAUC	GTGAAAGCCA(217.18 187.18 187.15	GAA.12 AGCACUU GAAAAGGAAGTGGTAAGTCTCAGGGGAG 3' UUUCCUUCACCAUUCAGA UUCCUUCACCAUUCAGAG CCUUCACCAUUCAGAG
TATAGCAGCCTCTGCAACAC AUAUC	GTGAAAGCCA 217.18 187.18 187.15 187.12	GAA.12 AGCACUU GAAAAGGAAGTGGTAAGTCTCAGGGGAG 3' UUUCCUUCACCAUUCAGA UUCCUUCACCAUUCAGAG CCUUCACCAUUCAGA CUUCACCAUUCAGA
TATAGCAGCCTCTGCAACAC AUAUC	GTGAAAGCCA 217.18 187.18 187.15 187.12 187.10	GAA.12 AGCACUU GAAAAGGAAGTGGTAAGTCTCAGGGGAG 3' UUUCCUUCACCAUUCAGA UUCCUUCACCAUUCAGAG CCUUCACCAUUCAGA CUUCACCAUUCA CUUCACCAUU
TATAGCAGCCTCTGCAACAC AUAUC	GTGAAAGCCAC 217.18 187.18 187.15 187.12 187.10 164.18	GAA.12 AGCACUU GAAAAGGAAGTGGTAAGTCTCAGGGGAG 3' UUUCCUUCACCAUUCAGA UUCCUUCACCAUUCAGAG CCUUCACCAUUCAGA CUUCACCAUUCA CUUCACCAUU UCCUUCACCAUUCAGAGU
TATAGCAGCCTCTGCAACAC AUAUC	GTGAAAGCCAC 217.18 187.18 187.15 187.12 187.10 164.18 188.18	GAA.12 AGCACUU GAAAAGGAAGTGGTAAGTCTCAGGGGAG 3' UUUCCUUCACCAUUCAGA UUCCUUCACCAUUCAGAG CCUUCACCAUUCAGA CUUCACCAUUCA CUUCACCAUUCAGAGU CCUUCACCAUUCAGAGU
TATAGCAGCCTCTGCAACAC	GTGAAAGCCAC 217.18 187.18 187.15 187.12 187.10 164.18 188.18 218.18	GAA.12 AGCACUU GAAAAGGAAGTGGTAAGTCTCAGGGGAG 3' UUUCCUUCACCAUUCAGA UUCCUUCACCAUUCAGAG CCUUCACCAUUCAGAA CUUCACCAUUCAGAGU UCCUUCACCAUUCAGAGU CCUUCACCAUUCAGAGUC CUUCACCAUUCAGAGUCC

Supplemental Figure 1. Schematic drawing of the *BTK* sequence and the tested SCOs. The mutation (A to T: nucleotide in green) causes the aberrant splicing by creating a novel 5' splice-site. This leads to activation of an upstream cryptic 3' splice-site and to the inclusion of a pseudoexon (sequence in red) is depicted. In yellow are the SCOs designed to target the pseudoexon 3' and 5' splice-sites and putative exonic splicing-enhancers (ESEs). The modifications of these SCOs are indicated in Table 1. Note that SCOs 191.18 and GAA.12 are interrupted in the figure.



Supplemental Figure 2. Splice-correction-induced up-regulation of reporter mini-gene activity using further modified SCOs. Efficacy of modified SCOs from the 186-, 187- and GAA- series following transfection at low doses as measured by the restoration of *BTK* mini-gene. A concentration of 30 nM was used for the scrambled control SCO (Scr) (Table 1). A representative gel from two independent experiments is shown. NT: non-treated cells.



Supplemental Figure 3. Semi-quantitative RT-PCR identifying splice-correction in the minigene reporter cell line. Modified SCOs from the 186- and 187-series were compared by transfection at low doses. A representative gel from two independent experiments is shown.



Supplemental Figure 4. Selective detection of human *BTK* **mRNA in a human BAC-transgenic mouse model.** RT-PCR of total RNA isolated from splenic B cells of BAC-transgenic, WT, or *Btk* KO mice, or from blood samples of a healthy control subject or a healthy carrier (XLA patient's sister). Two of the B cell samples were stimulated with CpG ON only or CpG ON together with anti-IgM in order to promote survival. The primer set was designed to only detect human *BTK* RNA, i.e. only human BAC-transgenics will generate amplicons by this assay. Since the disorder is X-linked, a healthy female carrier (here the patient's sister) will have two copies of the gene, one mutated and one normal, yielding both the aberrant and the correct mRNA species from a peripheral blood sample containing polyclonal BTK-expressing hematopoietic cells. NTC: non-treated water control.



B



Supplemental Figure 5. Flow cytometric analysis showing CLPs (common lymphoid progenitors) (A) and B-cell progenitors (B) in bone marrow from WT, *Btk* KO and BAC-transgenic mice. (A) CLPs (FLT3⁺IL7R⁺) are further subdivided based on LY6D to visualize the more B-cell specified LY6D⁺ CLPs. Right panel shows absolute number of indicated cell population per two femurs. (B) Sub-fractionation of CD19⁺ B-cells into Mature B (IgM⁺IgD⁺), Immature B (IgM⁺IgD⁻) and earlier progenitors based on BP1. Right panels show absolute number of indicated cell population per two femurs.



Supplemental Figure 6. Purity of pro-B cells. At the day of the transfection (day 9), the purity of the pro-B cells was assessed by CD19 staining and the viability of the cells was confirmed by PI staining. The purity and viability was checked for cells from duplicate animals with a representative result of each presented above.



Supplemental Figure 7. Restoration of BTK protein expression upon splice-correction after in vivo-treatment of BAC-transgenic mice. Western blot analysis of BTK restoration in two out of four treated animals; total cells from bone marrow and spleen (the other two treated animals are shown in Figure 8). Bar graph shows the quantitative analysis of BTK protein as percentage-relative intensity signal according to the ImageJ Software.