MLL-ENL leukemia burden initiated in femoral diaphysis and preceded by mature B-cell depletion

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Supplementary Design and Methods

In vivo *imaging*

In vivo imaging was performed as described[1] or animals were euthanized at different time points; tibias of transplanted mice were removed and the femurs were superficially pierced with a 23-gauge needle through the joint. Through this route, the S300 flexible microprobe (Mauna Kea Technologies™), containing 10,000 optical fibers that carry light from a continuous laser source at 488 nm to the living tissue, was entered and moved slowly inside the femoral cavity all the way up to the femoral head. Fluorescence emitted by excitation of the GFP protein is carried back by optical fibers to the apparatus where a dedicated set of algorithms reconstructs images in real time. The rate of acquisition is 12 frames per second and the S-300 flexible microprobe has a field of the reconstituted image covering a 300 μm circular diameter in a focal plane, 0-15 μm away from the probe's optical window with a spatial resolution in the plane of 3.3 μm compatible with the size of single cells (8-10 μm). The flexible probe was entered at the level of the knee and then moved slowly (0.3-0.6 mm per second) inside the femoral cavity, all the way up to the femoral head. The complete femoral canal of a mouse was scanned in 30-50 s at a constant displacement speed.[1] S1500 flexible macroprobe was used for imaging the surface of the organs. Video data acquisitions and analyses were performed with the CellVizio 488® and Image Cell software (Mauna Kea Technologies™, France) (*Supplementary video 1*).

References

1 Lewandowski D, Barroca V, Ducongé F, Bayer J, Van Nhieu JT, Pestourie C, et al. In vivo cellular imaging pinpoints the role of reactive oxygen species in the early steps of adult hematopoietic reconstitution. Blood. 2010;115(3):443-52.

Online Supplementary Table S1. Total number of hematopoietic cells in the three femoral compartments.

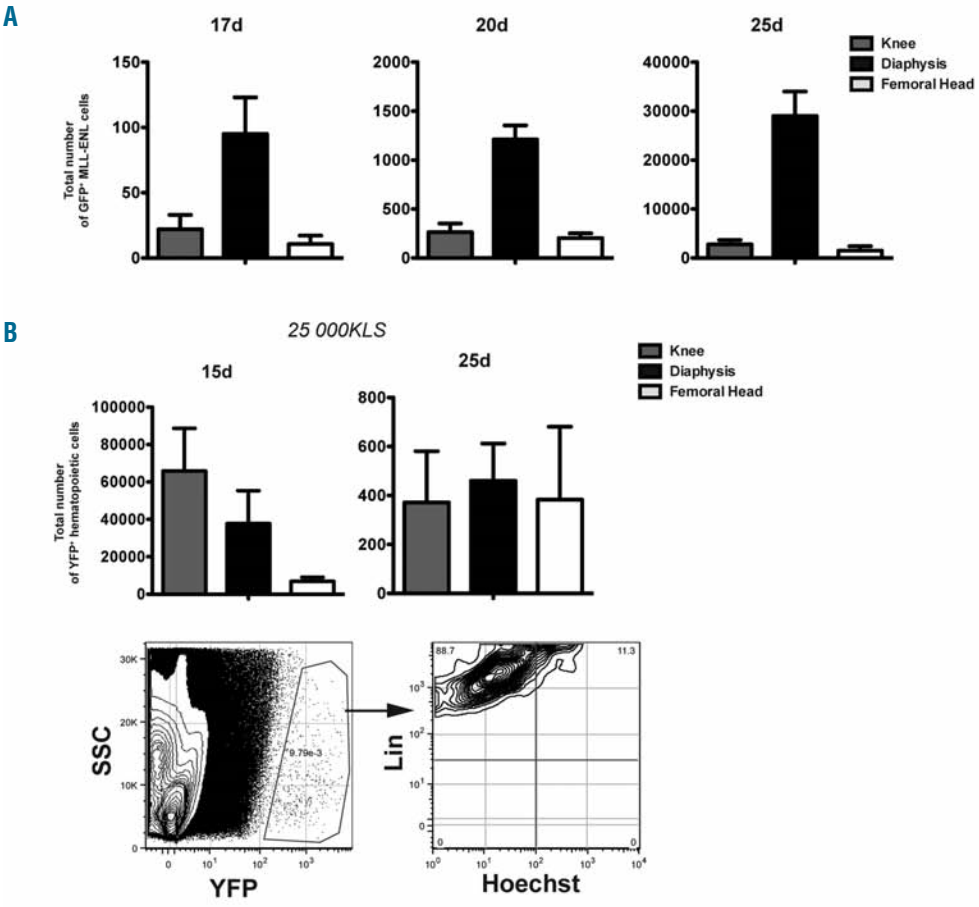
	Knee	Diaphysis	Femoral Head
Total number of hematopoietic cells	$4.2x10^6$	$10.1x10^6$	$1.6x10^6$
	$\pm 0.7 x10^6$	$\pm 1.2 x10^6$	$\pm 0.4 x10^6$

Total number of hematopoietic cells from femoral head, diaphysis and knee of non-conditioned 129/Sv mouse. Data represent mean ± SEM of hematopoietic cells from the two femurs of non-conditioned 129/Sv mice (n=10).

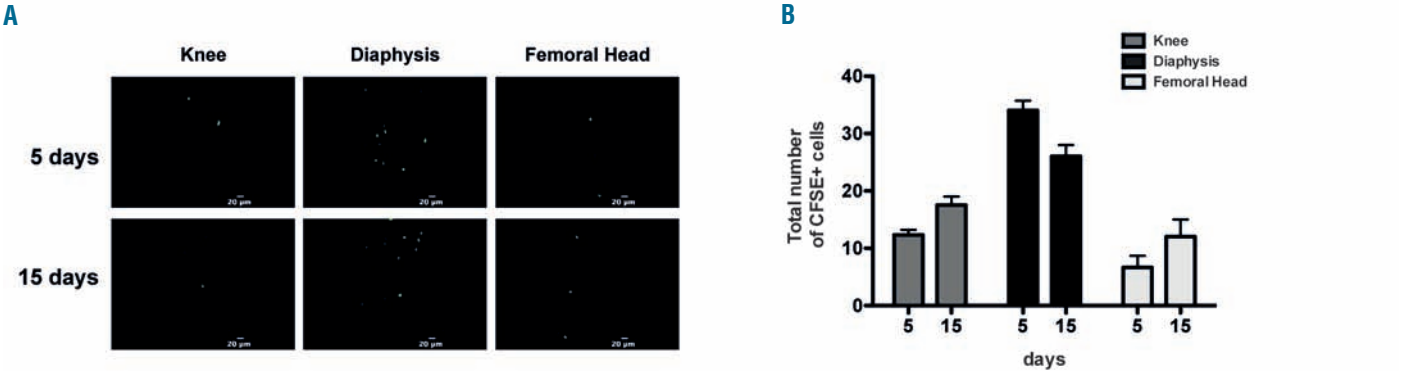
Online Supplementary Video S1. Technique used to insert the flexible probe at the level of the knee and to move the probe slowly inside the femoral cavity. SEE ATTACHED MOVIE

Online Supplementary Video S2. Twenty-five days after injection of 1,000 GFP+ leukemic cells into non-conditioned 129/Sv mice, video footage of *in vivo* imaging of the femoral cavity shows many GFP+ leukemic cells in clusters in the diaphysis and very few single cells in the femoral head and knee's epiphyses. The position of the tip of the S300 flexible microprobe in the femoral cavity is illustrated in parallel to the video in the upper right. SEE ATTACHED MOVIE

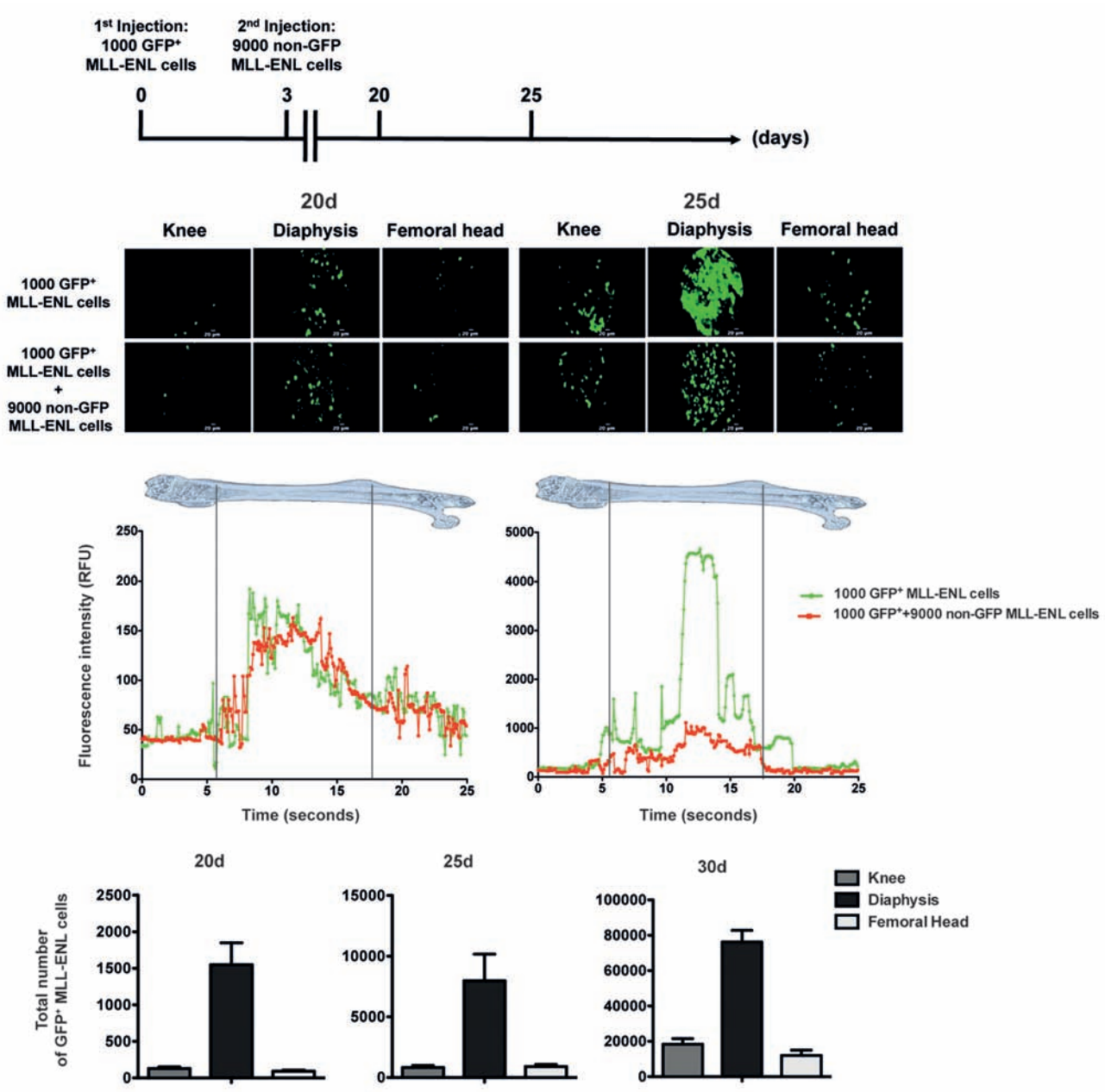
Online Supplementary Video S3. One thousand GFP+ leukemic cells were injected into non-irradiated 129/Sv mice and challenged, 3 days after, by a second injection of 9000 GFP- leukemic cells. Twenty-five days after injection, video footage of *in vivo* imaging of the femoral cavity shows few GFP+ leukemic cells in clusters and many of them in single cells in the diaphysis and very few single cells in the femoral head and knee's epiphyses. The position of the tip of the S300 flexible microprobe in the femoral cavity is illustrated in parallel to the video in the upper right. SEE ATTACHED MOVIE



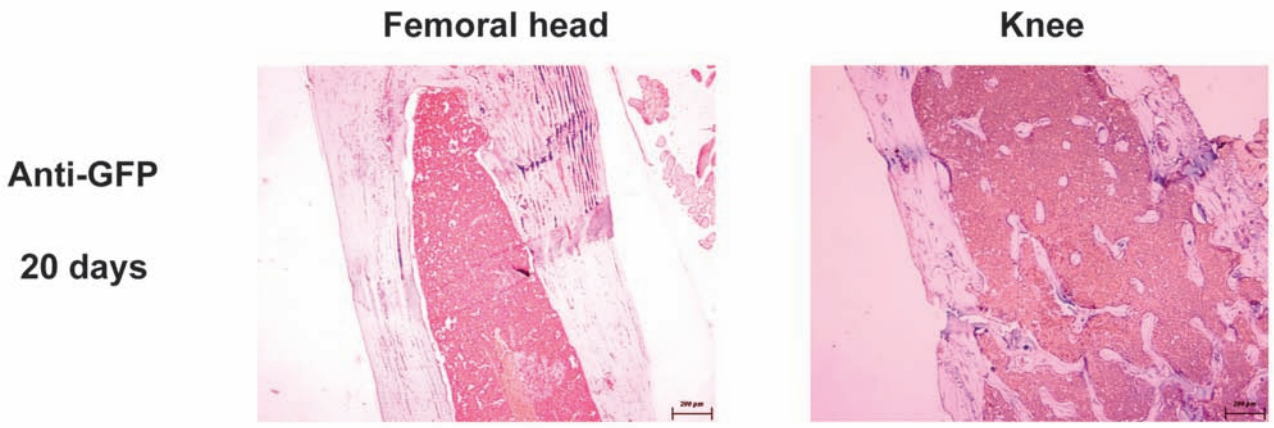
Online Supplementary Figure S1. (A) One thousand GFP$^+$ MLL-ENL leukemic cells were injected into non-conditioned 129/Sv syngenic mice by the jugular vein route. Seventeen, 20, and 25 days after injection, percentages of GFP$^+$ leukemic cells located in femoral head, diaphysis and knee were determined by flow cytometry. Histograms show the total number of GFP$^+$ leukemic cells for each femoral compartment at the indicated day. These numbers were obtained by multiplying cell numbers of the three femoral compartments by the percentage of GFP$^+$ leukemic cells of each femoral compartment and represent mean±SEM (n=5). (B) Upper panel: non-conditioned 129/Sv recipient mice were intravenously transplanted with 25,000 YFP$^+$ KLS hematopoietic cells. Fifteen and 25 days after transplantation, percentages of YFP$^+$ hematopoietic cells located in femoral head, diaphysis and knee were determined by flow cytometry. Histograms show the total number of YFP$^+$ hematopoietic cells for each femoral compartment at the indicated day. These numbers were obtained by multiplying cell numbers of the three femoral compartments by the percentage of YFP$^+$ hematopoietic cells of each femoral compartment and represent mean±SEM (n=5). Lower panel: twenty five days after transplantation of 25,000 YFP$^+$ KLS hematopoietic cells, percentages of immature (Lin$^-$) and mature (Lin$^+$) YFP$^+$ hematopoietic cells were determined and FACS diagrams show the Lineage positive YFP$^+$ hematopoietic cells in femurs (n=5). (C) Ten thousand GFP$^+$ MLL-ENL leukemic cells were injected into non-conditioned 129/Sv syngenic mice through the jugular vein. Seventeen, 20, and 25 days after injection, percentages of GFP$^+$ leukemic cells located in femoral head, diaphysis and knee were determined by flow cytometry. Histograms show the total number of GFP$^+$ leukemic cells for each femoral compartment at the indicated days. These numbers were obtained by multiplying cell numbers of the three femoral compartments by the percentage of GFP$^+$ leukemic cells of each femoral compartment and represent mean±SEM (n=5).



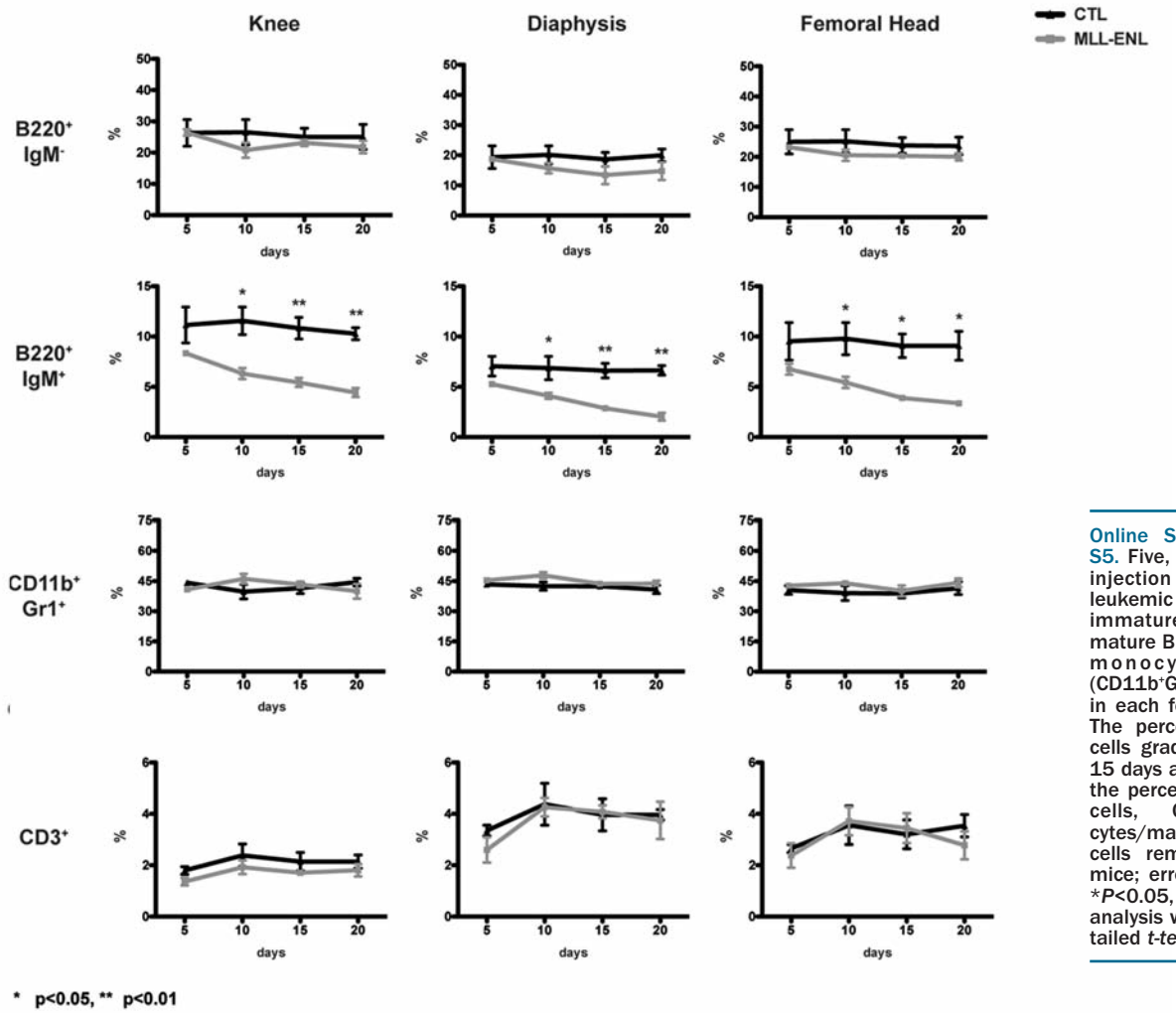
Online Supplementary Figure S2. (A) Five and 15 days after injection, intra-femoral videos were performed to track CFSE labeled leukemic cells. Pictures represent frames of 20s to 35s videos acquired over the length of the femur and show CFSE labeled leukemic cells in knee, diaphysis and femoral head (scale bar 20 μm). The data are representative of 3 experiments with 5 mice per day of acquisition. (B) Quantification, by FACS analysis, of the number of CFSE labeled leukemic cells present in knee, diaphysis and femoral head of non-conditioned 129/Sv mice injected with 1,000 CFSE-labeled MLL-ENL leukemic cells. Data represent mean±SEM of CFSE-labeled leukemic cells found in each femoral compartment of 5 different transplanted mice at each indicated day.



Online Supplementary Figure S3. Kinetics of GFP+ leukemic cell femoral development when challenged by non-labeled leukemic cells. One thousand GFP+ MLL-ENL leukemic cells were injected into non-conditioned 129/Sv mice and challenged, 3 days after, by a second injection of 9,000 non-labeled MLL-ENL leukemic cells. Upper panel: schematic drawing of the delayed competition experiment. Middle panel: twenty and 25 days after single or competitive transplantation, video data were acquired. Pictures are representative of the distribution of GFP+ MLL-ENL leukemic cells inside the knee, diaphysis and femoral head areas. The graphics shown below represent the mean fluorescence (RFU) detected across the femur by the 10,000 optical fibers per individual video frame for a single (green line) or a competitive (red line) injection. Lower panel: histograms show total number of GFP+ leukemic cells for each femoral compartment 20, 25 and 30 days after the injection of 1,000 GFP+ leukemic cells challenged 3 days after with 9,000 non-labeled competitor leukemic cells. These numbers were obtained by multiplying cell numbers of the three femoral compartments by the percentage of GFP+ leukemic cells of each femoral compartment and represent mean±SEM (n=5 for each indicated day).



Online Supplementary Figure S4. Immunohistochemistry of GFP$^+$ leukemic cells in knee and femoral head of femurs of 129/Sv mice 20 days after injection of 1,000 GFP$^+$ leukemic cells. No GFP$^+$ leukemic cells were observed in knee and femoral head compared to diaphysis where we detected clusters of leukemic cells (see main text Figure 4).



Online Supplementary Figure S5. Five, 10 and 15 days after injection of 1,000 GFP$^+$ leukemic cells, percentages of immature (B220$^+$IgM$^-$) and mature B cells (B220$^+$IgM$^+$) and monocytes/granulocytes (CD11b$^+$Gr1$^+$) were determined in each femoral compartment. The percentage of B220$^+$IgM$^+$ cells gradually decreases 5 to 15 days after injection whereas the percentage of B220$^+$IgM$^-$ B cells, CD11b$^+$Gr1$^+$ monocytes/macrophages and CD3$^+$ T cells remain constant (n=4 mice; error bars indicate SEM, *P<0.05, **P<0.01). Statistical analysis was performed by two-tailed *t-test*.