Supplementary Information for

Mast cells rescue implantation defects caused by c-kit deficiency

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This file contains Supplementary Tables S1 and S2 and Supplementary Figures S1-S16

Supplementary Table S1. Implantation numbers C57BL/6 (n=8) and *Kit*$^{W\text{-}sh/W\text{-}sh}$ (n=8) females after syngeneic mating with C57/BL6 males (Mann Whitney-*U* test)

Syngeneic matings	Implantations (mean)
C57/BL6 x C57/BL6 (n=8)	9.57
C57BL/6J-Kit w-sh/w-sh x C57/BL6 (n=8)	5.25* ($p < 0.05$)

Supplementary Table S2. Litter size from C57BL/6 (n=7), *Kit*$^{W\text{-}sh/W\text{-}sh}$ (n=8) and *Kit*$^{W\text{-}sh/W\text{-}sh}$ reconstituted with BMMCs (n=10) after allogeneic mating with BALB/c males (Mann Whitney-*U* test)

	Pups/mother (mean; median)
C57BL/6J	44/7 (5.5; 7)
W-sh	23/8 (2.9; 0)
W-sh+BMMC	73/10 (7.4; 9.5)

Supplementary Figure Legends

Figure S1: **Number of follicles and corpora lutea in ovaries of C57BL/6J mice (n=4),** $Kit^{W\text{-}sh/W\text{-}sh}$**(n=5) and** $Kit^{W\text{-}sh/W\text{-}sh}$ **mice reconstituted with BMMCs (n=6).** Paraffin-embedded ovaries were cut and stained with H&E for analysis of follicles and corpora lutea as described by Gartner and Hiatt (Color Textbook of Histology, 2001). No statistically significant differences could be detected in the number of primary (A), secondary (B) and tertiary (C) follicles among the groups as analyzed by the Mann-Whitney-U-test. Similarly, the number of corpora lutea was similar among the groups (D), indicating that all animals ovulate normally. The number of follicles was analyzed in the left and right ovaries in each animal and the mean of each specimen was used to determine the mean of each group. Data are shown as mean ± S.E.M. One representative picture of either a follicle or a corpus luteum is depicted close to the graphic.

Figure S2: **Uterine mast cells as analyzed by flow cytometry.** Total uterine cells were isolated from wild type mice using enzymatic digestion showing the gate of lymphocytes.(A) shows the percentage of cells positive for Mcpt8. (B) Uterine MCs positive for CD117 and $FcR\varepsilon^{+}$ cells within the gated cells. These cells constitute around 20% of the gated immune cells (in this representative example 22.3%). (C) Percentage of EYFP positive cells within the uterine MC population as analyzed using Mcpt5.ROSA.cre.EYFP animals in which $Mcpt5^{+}$ cells are positive for EYFP. It is important to note that only 4.49% of the uterine MCs are positive for Mcpt5.

Figure S3: ***Unilateral local reconstitution with BMMCs restores the rate of implantations in MC-deficient mice.*** Representative images of uteri from a virgin C57BL/6J female, a normal pregnant C57BL/6J mouse, a non-pregnant $Kit^{W\text{-}sh/W\text{-}sh}$ (W-sh) female with an inflamed and thickened uterus after successful mating and a pregnant $Kit^{W\text{-}sh/W\text{-}sh}$ female which was locally reconstituted with BMMCs. Although more blastocysts were implanted in the reconstituted uterine horn at least one implantation could be found in the mock-treated site close to the reconstituted site.

Figure S4: ***Unilateral local reconstitution of MC-deficient mice with BMMCs induces upregulation of Mcpt-1, -5 and -8 in the decidua on day 10 of pregnancy.*** mRNA levels of Mcpt-1 (*A*), Mcpt-5 (*B*) and Mcpt-8 (*C*) in the left (L) and right (R) uterine horns of non-pregnant $Kit^{W\text{-}sh/W\text{-}sh}$ females (W-sh; n=3), in left (L) and right (R) whole implantation sites of pregnant $Kit^{W\text{-}sh/W\text{-}sh}$ females (n=3), as well as in locally reconstituted $Kit^{W\text{-}sh/W\text{-}sh}$ mice (n=7). After reconstitution, MC-related genes were significantly augmented compared to controls.

The higher expression of MC proteases in pregnant *Kit*$^{W\text{-}sh/W\text{-}sh}$ animals compared to non-pregnant *Kit*$^{W\text{-}sh/W\text{-}sh}$ is explainable by the nature of the tissue. Whole implantation sites (placenta + fetus + decidua) were used for RNA isolation. Fetal tissue (placenta and fetus) is not deficient in MCs. Data are expressed as median. $P<0.05$: *, $p<0.1$: #, as analyzed by Wilcoxon test between left and right from the same group and Mann Whitney-*U* test between different groups.

Figure S5*: *MCs localize in both reconstituted and non-reconstituted uterine horns following local BMMC reconstitution of Kit*$^{W\text{-}sh/W\text{-}sh}$ *mice. BMMCs were injected locally into the right uterine horn of *Kit*$^{W\text{-}sh/W\text{-}sh}$ female mice (n=10), whereas their left horn was treated with PBS. The middle photograph depicts both uterine horns as registered on day 10 of pregnancy. Six implantations were observed at the reconstituted horn (A) and four in the non-reconstituted one (B). The presence of MCs in decidual tissue from both, reconstituted and non-reconstituted horn was confirmed by Toluidine blue O staining (0.1%). Photographs are shown using a total magnification of 1000X.

Figure S6: shows implantations from different sizes as analyzed by H&E staining at day 5 of pregnancy.

Figure S7: ***mRNA uterine levels of uPA, tPA, VEGF, MMP-9 and mPAI-1 after restored implantation following systemic reconstitution with BMMCs***. The figure illustrates increased levels of uPA and tPA, but not of VEGF-A, MMP-9 and PAI-1 at the fetal-maternal interface of *Kit*$^{W\text{-}sh/W\text{-}sh}$ MC-deficient mice (W-sh) systemically reconstituted with BMMCs. Expression of uPA (A), tPA (B), VEGF-A (C), MMP9 (D) and PAI-1 (E) mRNA in decidual tissue of female C57BL/6J (n=15-16), non-reconstituted *Kit*$^{W\text{-}sh/W\text{-}sh}$ (n=13-14) and systemically reconstituted *Kit*$^{W\text{-}sh/W\text{-}sh}$ (n=14) mice. Although the expression of VEGF-A, MMP-9 and PAI-1 is augmented in systemically reconstituted W-sh as compared to their not reconstituted counterpart, expression of these mediators is also higher in non-reconstituted *Kit*$^{W\text{-}sh/W\text{-}sh}$ versus wild-type controls which show normal implantations. Data are expressed as single dots with medians. #:$p<0.1$, *:$p<0.05$, **:$p<0.005$, ***: $p<0.001$ as analyzed by Kruskall-Wallis test followed by Mann-Whitney-*U* test.

Figure S8: ***Mast cell proteases -1, -5 and -8 are present in decidual tissue of MC-deficient mice following systemic reconstitution with BMMCs.*** The chymases Mcpt-1 (A), -5 (B) and -8 (C) were analyzed at the mRNA level in C57BL/6J (n=15), *Kit*$^{W\text{-}sh/W\text{-}sh}$ (W-sh; n=14) and *Kit*$^{W\text{-}sh/W\text{-}sh}$ systemically reconstituted with wt-BMMC (n=14). Reconstitution with BMMCs resulted in an increase of MC proteases within the decidua of *Kit*$^{W\text{-}sh/W\text{-}sh}$ females.

Data are expressed as single dots with medians and analyzed by Kruskall-Wallis test followed by Mann-Whitney-*U* test. ***:$p<0.001$.

Figure S9: Correlation between TGF-β1, CtGF and MC-proteases (A) The amounts of TGF-β1 correlate with Mcpt-8 levels in uterine tissue (p: 0.0073). (B) The amounts of CtGF correlate with Mcpt-8 levels (p: 0.022). (C) The amounts of TGF-β1 correlate with CtGF levels in uterine tissue (p: 0.022). The expression of these molecules was analyzed by Real Time PCR. Correlations were analyzed by Pearson.

Figure S10: Giant cells from MC-deficient mice reconstituted with Lgals1$^{-/-}$BMMCs show abnormal morphology. Paraffin-embedded sections (5 µm) from placentas were stained with hematoxylin/eosin to evaluate their morphology. *Kit$^{W\text{-}sh/W\text{-}sh}$* (W-sh) mice systemically reconstituted with *Lgals1$^{-/-}$* BMMCs developed abnormal placentas characterized by atypical giant cells (GC) preseting vacuoles inside their cytoplasm (B) after allogenic mating with BALB/c males on day 10 of pregnancy compared to the normal structure of GCs in wild-type mice or MC-deficient mice reconstituted with wt BMMCs (A). Photographs are shown using a total magnification of 200X.

Figure S11: ***Transfer of Lgals1$^{-/-}$ BMMCs into Kit$^{W\text{-}sh/W\text{-}sh}$*** **mice resulted in incomplete reconstitution.** *Kit$^{W\text{-}sh/W\text{-}sh}$* (W-sh) mice transferred with *Lgals-1$^{-/-}$* or wild type BMMCs were analyzed for the frequency of CD117^{+}/Fc**ε**R^{+} cells in inguinal (A), mesenteric (B) and para-aortic (C) lymph nodes as well as in the decidua (D). Reconstitution was incomplete in decidua and the draining lymph nodes. Data are expressed as single dots with medians and analyzed by Kruskall-Wallis test followed by Mann-Whitney-*U* test. *:$p<0.05$ and *:$p<0.01$.

Figure S12: Lgals1 defective BMMCs did not proliferate properly in the presence of trophoblasts: Wild type or *Lgals1* defective BMMCs were cultured either alone or in the presence of SM-9 trophoblasts for 48 h. When lacking Gal-1, BMMCs did not proliferate in the same extent as wild type BMMCs did. **:p<0.01 as analyzed by unpaired t test. Data are the mean of three independent experiments.

Figure S13: Pregnant Lgals1$^{-/-}$ mice show suboptimal spiral artery formation which could be restored by wild type MCs. Spiral arteries were evaluated regarding their morphology. The lumen diameter (C) was measured and wall thickness (B) of 3-8 spiral arteries per female and the wall:lumen ratio (A) was calculated by ImageJ and the mean was included in the graph. Lack of Gal-1 led to impaired spiral artery formation as well as increased placenta size all of which was normalized after transfer of wild type BMMCs.

Representative pictures are shown in D-F (magnification 400X). Data are expressed as means. Statistical significances were analyzed by unpaired *t*-test. **:*p*<0.005, ***: *p*<0.001

Figure S14: ***Implantations (day 5) are smaller in mice lacking Gal-1 (Lgals1$^{-/-}$) as compared with their wild-type counterparts.*** Chicago blue dye was injected i.v. into C57BL/6J (n=9), *Kit*$^{W\text{-}sh/W\text{-}sh}$ (W-sh; n=7) and *Lgals1*$^{-/-}$ (n=7) mice for a better visualization of the implantation sites on day 5 of pregnancy. One to seven implantations per female were measured by ImageJ, the mean was calculated and included in the graph. When Gal-1 or MCs were absent, the size of the implantations on day 5 of pregnancy was found to be significantly reduced as compared to C57BL/6J females. Data are expressed as mean. Statistical significances were analyzed by unpaired *t*-test. *P*<0.005: **.

Figure S15: ***Purity of cultured BMMCs.*** Representative dot plot from cultured BMMCs used for systemic and local transfer. Cells were stained with antibodies against CD117 and FCεRI. In this culture, BMMCs showed a purity of 97.52% after 37 days of culture, which is representative of all staining performed after BMMC culture.