Supplementary material

Tracking mesenchymal stem cell contributions to regeneration in an immunocompetent cartilage regeneration model

Daniela Zwolanek, María Satué, Verena Proell, Josè R. Godoy, Kathrin I. Odörfer, Magdalena Flicker, Sigrid Hoffmann, Thomas Rülicke, and Reinhold G. Erben

Corresponding Author

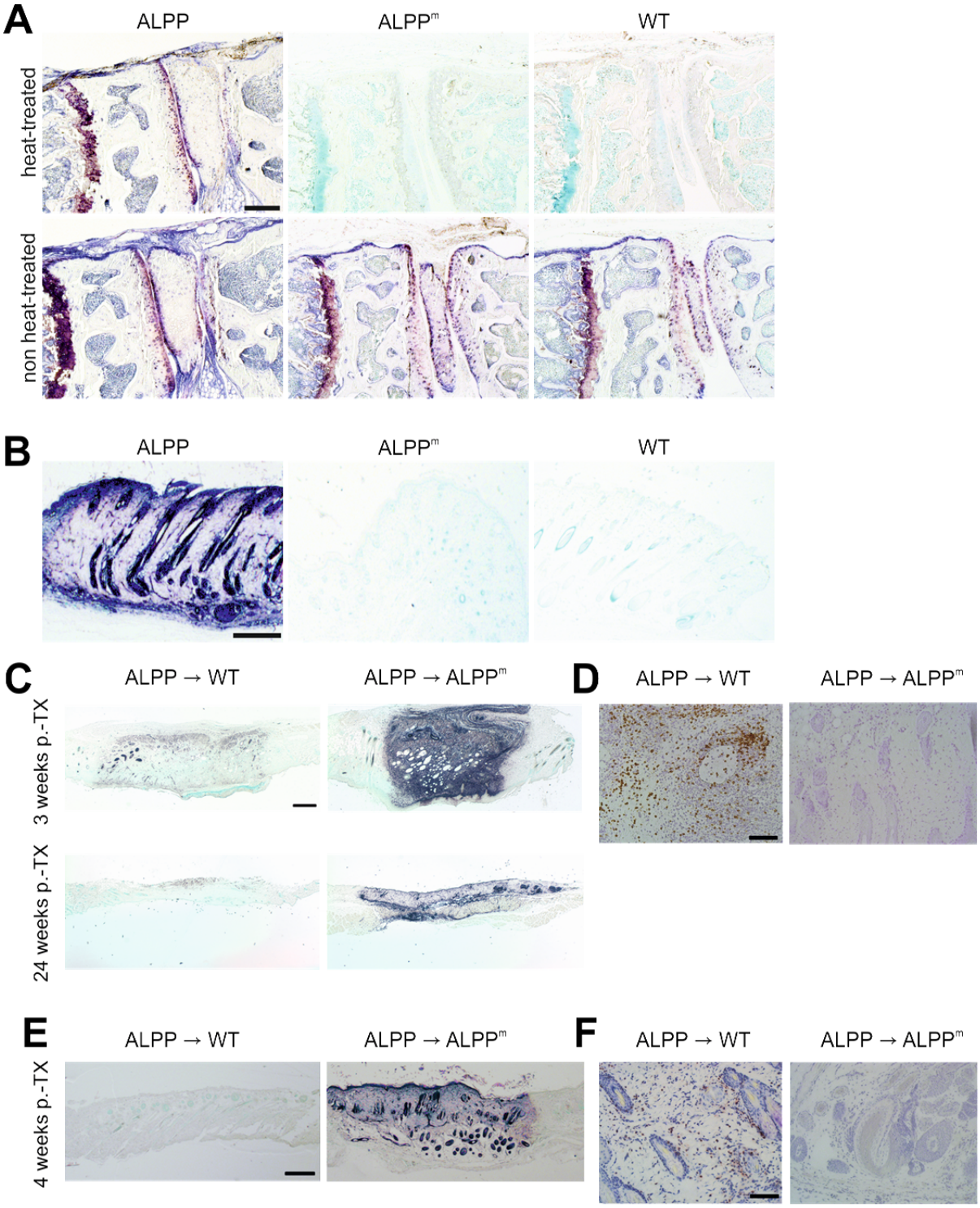
Reinhold G. Erben, MD, DVM

Institute of Physiology, Pathophysiology, and Biophysics

Department of Biomedical Sciences, University of Veterinary Medicine Vienna

Veterinärplatz 1, 1210 Vienna, Austria, Tel: +43-1-250 77-4550, Fax: +43-1-25077-4599,

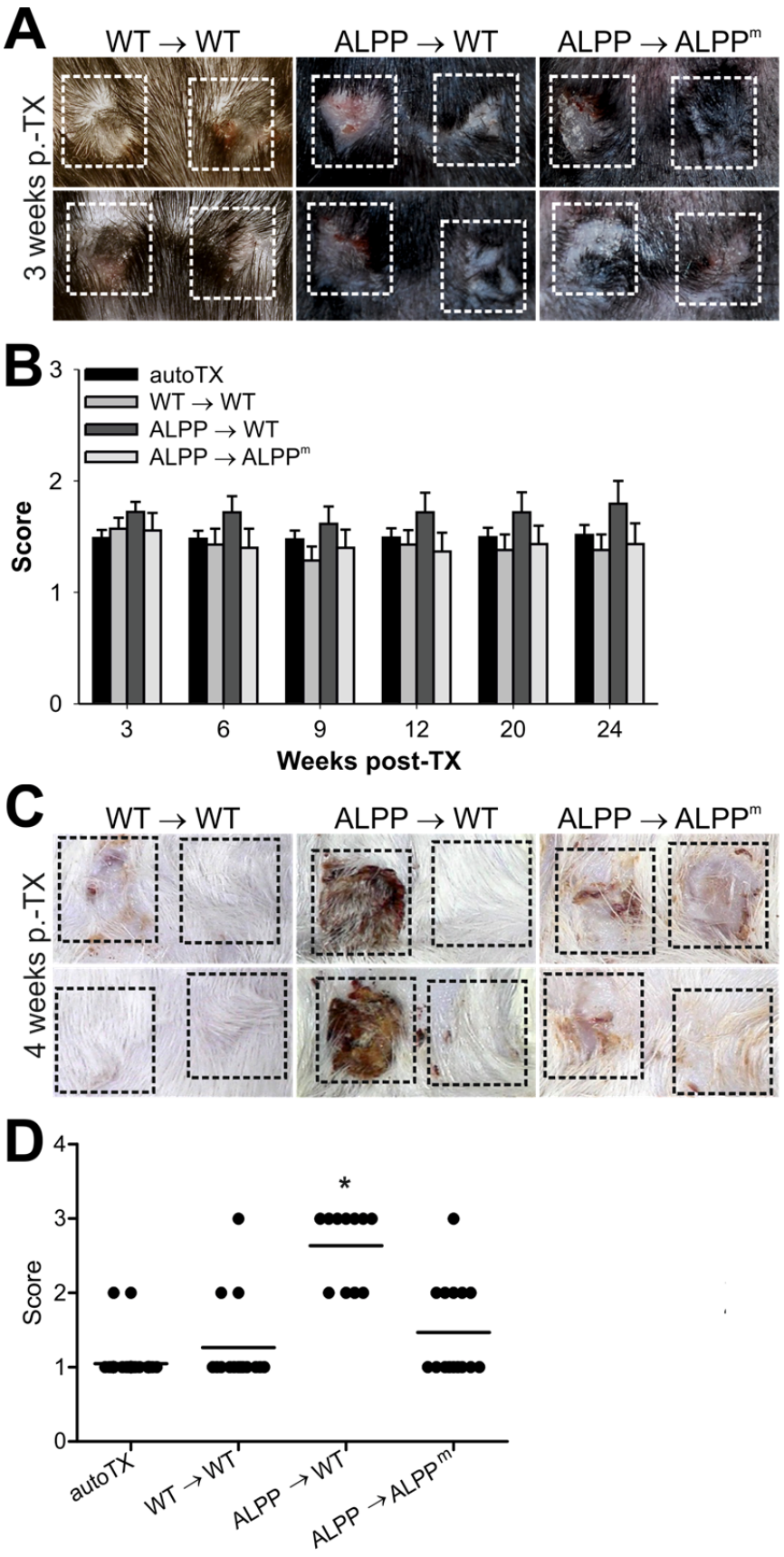
E-mail: Reinhold.Erben@vetmeduni.ac.at



Supplementary Figure 1, Zwolanek *et al.*

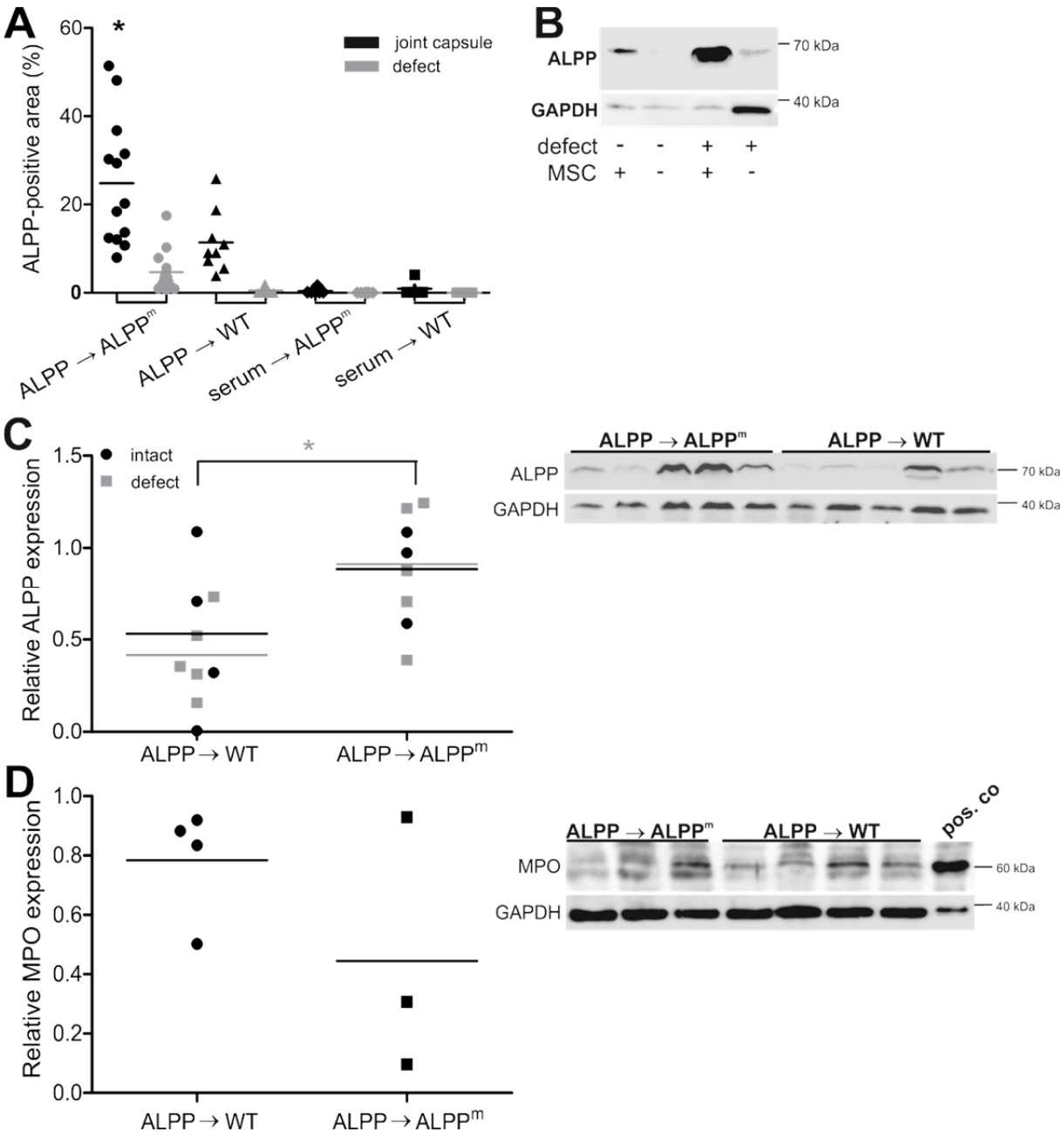
Supplementary Figure S1. Histochemical ALPP staining and long-term tolerance of Tg(ALPP^{m}) recipients to skin grafts from Tg(ALPP) donors. (**A**) ALPP histochemistry on plastic sections from knee joints of Tg(ALPP), Tg(ALPP^{m}), and wild-type mice after a 35-min heat inactivation at 72°C (upper panels) or without heat pretreatment (lower panels). Tg(ALPP) donors show strong staining in all tissues, whereas no enzyme activity could be detected in wild-type and Tg(ALPP^{m}) mice upon heat pretreatment. Non heat-treated tissues

show similar enzyme activity in all mouse lines. Scale bar = 100 µm. n = 3 per group. (**B**) ALPP histochemistry of skin sections from Tg(ALPP), Tg($ALPP^m$) and wild-type rats after 35 min heat inactivation at 72°C. Strong ALPP staining was present in Tg(ALPP) rats compared to Tg($ALPP^m$) and wild-type littermates. Scale bar = 100 µm. n =5 per group. (**C**) Histochemistry of skin grafts from Tg(ALPP) grafts transplanted into Tg($ALPP^m$) recipient mice showing more host tissue as shown in Figure 2B. Scale bar = 500 µm. n = 10 per group. (**D**) Immunohistochemical staining of skin grafts from wild-type and Tg($ALPP^m$) recipient mice using an anti-CD45R antibody, 3 weeks post-surgery. Red-stained cells represent CD45R-positive leukocytes. Scale bar = 100 µm. n ≥ 5 per group. (**E**) Histochemistry of skin grafts from Tg(ALPP) rats transplanted into Tg($ALPP^m$) recipient rats showing more host tissue as shown in Figure 2D. Scale bar = 500 µm. n = 10 per group. (**F**) Immunohistochemical staining of skin grafts from wild-type and Tg($ALPP^m$) recipient rats using an anti-CD45 antibody, 4 weeks post-surgery. Red-stained cells represent CD45-positive leukocytes. Scale bar = 100 µm n ≥ 5 per group. $ALPP^m$, $ALPP^{E451G}$ mutant; *ALPP* → $ALPP^m$, transplantation from Tg(ALPP) donor into Tg($ALPP^m$) recipient; TX, transplantation; WT, wild-type.



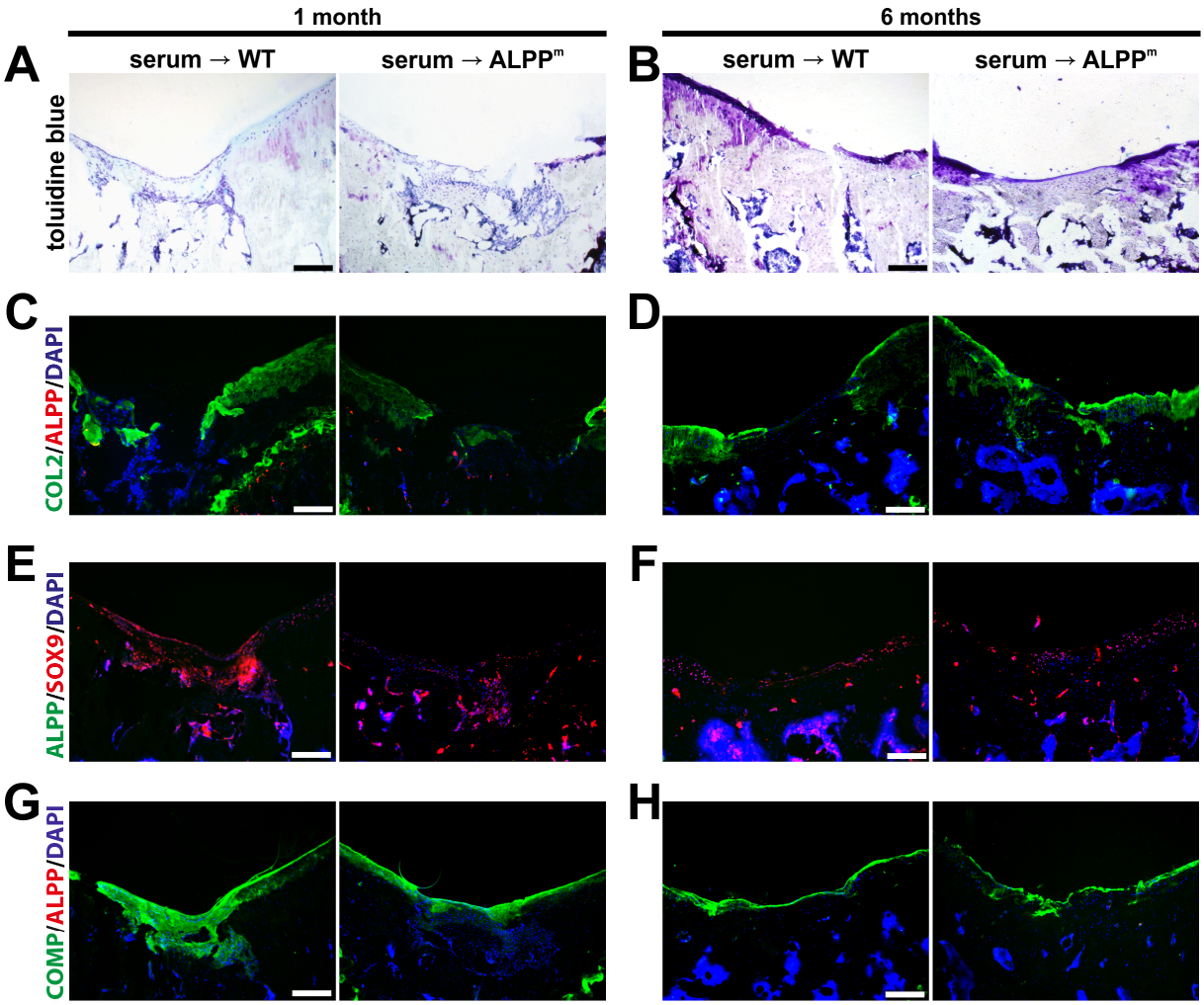
Supplementary Figure S2. Macroscopic analysis of skin grafts from Tg(ALPP) donors in mouse and rat marker-tolerant models. (**A**) Macroscopic appearance of skin grafts (white dashed box) of indicated recipient mice, 3 weeks post-transplantation. The left dashed box indicates the donor transplant; the right box indicates the auto-transplant. (**B**) Macroscopic

scoring of skin graft rejection over the 24-week experimental period in mice. No significant differences were observed in the scoring of the transplants. Dots represent score per animal. n ≥ 10 per group. Data represent means ± SEM. (**C**) Macroscopic appearance of skin grafts (black dashed box) of indicated recipients in the rat model, 4 weeks post-transplantation. The left dashed box indicates the donor transplant; the right box indicates the auto-transplant. (**D**) Macroscopic scoring of skin graft rejection in the rat model, showing clear signs of rejection in wild-type recipients. Dots represent score per animal. n = 10 per group. * $p < 0.05$ by Kruskal-Wallis test followed by Mann-Whitney U-test. $ALPP^{m}$, $ALPP^{E451G}$ mutant; *ALPP* → $ALPP^{m}$, transplantation from Tg(ALPP) donor into Tg($ALPP^{m}$) recipient; TX, transplantation; WT, wild-type.



Supplemental Figure S3. Rapid rejection of MSC from Tg(ALPP) donors in the knee joint of wild-type recipient rats. (**A**) Quantification of the ALPP-positive area in the synovium of the joint capsule and within the full-thickness defects after histochemical ALPP staining, 1 day after intra-articular injection of serum or 10^7 MSC from Tg(ALPP) donor rats into the knee of wild-type and Tg(ALPP^m) recipient rats. Dots represent ALPP-positive area per animal. $n \geq 5$ per group. * $p < 0.05$ vs. WT recipients by Kruskal-Wallis test followed by Mann-Whitney U-test. (**B**) Western blotting analysis of joint capsule extract 1 day after injection of serum or 10^7 MSC from Tg(ALPP) donor rats into the knee of Tg(ALPP^m) recipients. GAPDH was used as loading control. $n \geq 5$ per group. (**C**) ALPP protein expression in joint capsule extracts from intact knees and knees carrying a cartilage defect, 1 day after intra-articular injection of MSC from Tg(ALPP) donor rats into Tg(ALPP^m) or wild-type recipients. GAPDH was used for normalization. * $p < 0.05$ by Student's t-test. $n = 5$ per group. (**D**) Western blotting analysis of MPO expression in joint capsule extracts, 1 day after intra-articular injection of MSC from Tg(ALPP) donor rats into Tg(ALPP^m) or wild-type recipi-

ents. GAPDH was used for normalization. Lysates of neutrophils served as positive control. n ≥ 3 per group. $ALPP^{m}$, $ALPP^{E451G}$ mutant; *ALPP* → $ALPP^{m}$, transplantation from Tg(ALPP) donor into Tg($ALPP^{m}$) recipient; TX, transplantation; MPO, myeloperoxidase; pos. co, positive control; WT, wild-type.



Supplementary Figure 4, Zwolanek *et al.*

Supplementary Figure S4. Serum controls for experiments with intra-articularly injected MSC in wild-type and Tg(ALPP^{m}) recipient rats. (**A and B**) Toluidine blue staining of cryosections from full-thickness cartilage defects in the patellar groove, 1 month (left panel) and 6 months (right panel) after injection of 50 µL rat serum into the knee joint of wild-type or Tg(ALPP^{m}) recipients. No neocartilage formation can be observed at 6 months post-treatment. Scale bar = 50 µm. n = 3 (A) and 5 (B) per group. (**C - H**) Immunofluorescence staining of cryosections of full-thickness cartilage defects, using anti-collagen II (green) and anti-ALPP antibodies (red) (**C and D**), anti-ALPP (green) and anti-Sox9 antibodies (red) (**E and F**), or anti-COMP (green) and anti-ALPP antibodies (red) (**G and H**), 1 month (left panels) and 6 months (right panels) after injection of rat serum into wild-type or Tg(ALPP^{m}) recipients. Neither COL2-containing cartilaginous neomatrix, nor SOX9 and COMP expression was detectable in the defects of serum-injected wild-type and Tg(ALPP^{m}) recipients, 6 months post-injection. No ALPP-expressing cells were found in Tg(ALPP^{m}) and wild-type recipients. Scale bar = 50 µm. n = 5 per group. ALPP, human placental alkaline phosphatase; ALPP^{m}, ALPP^{E451G} mutant; *ALPP* → ALPP^{m}, transplantation from Tg(ALPP) donor into Tg(ALPP^{m}) recipient; WT, wild-type.

Supplementary Table 1: PCR products after amplification

PCR product	Sequence (5' → 3')
R26S	GTCGACTAGATGAAGGAGAGC
E3r	GAGCCACATATGGGAAGCGGT
R26f	TGAATTCCTGCCTCGCCACTGT
U3Kr	GAAAGGAGCCTGCCTGGTACC
U3Kf	GGTACCAGGCAGGCTCCTTTC
3U1r	GAGGCAGAATCTCGCTCTGTC

Supplementary Table 2: PCR primers for cloning. *fw – forward, rev - reverse*

Primer	Direction	Use	Sequence (5' → 3')
SOE-f1	fw	ΔALPP cloning	TAGCACGTGGGAGACACTCCA
SOE-r1	rev	ΔALPP cloning	GTGCCCCTGGACGGAGAGACCCACGCAGGCGAGGAC
SOE-f2	fw	ΔALPP cloning	TGCGTGGGTCTCTCCGTCCAGGGGCACTGCTGACTG
SOE-r2	rev	ΔALPP cloning	GAAAGGAGCCTGCCTGGTACC
R26f	fw	genotyping	TGAATTCCTGCCTCGCCACTGT
E2r	rev	genotyping	AAGGCCTGGCTCACTCACCATC
U3f	fw	genotyping	GATGGAGACCATCCTGGCTAAC
U4r	rev	genotyping	GATCTAGTAACGGCCGCCAGTG
E5r	rev	screening	GCTACGCAGCTCATCTCCAA
E5f	fw	screening	GCTACGCAGCTCATCTCCAA
E9r	rev	screening	CTCTCAATGGCGTCGTCGAA
E8f	fw	screening	GATCCACCGAGACTCCACACT
E11r	rev	screening	AGGCCATGACGTGCGCTATGAA
U3f	fw	screening	GATGGAGACCATCCTGGCTAAC
U4r	rev	screening	GATCTAGTAACGGCCGCCAGTG

Supplementary Methods

Skin graft scoring. Scoring of grafts to evaluate rejection was performed on the basis of Hedrich (1) with minor modifications. Photographs of mouse allo- and autografts taken 3, 6, 9, 12, 20, and 24 weeks post-surgery and of rat grafts taken 4 weeks post-surgery were randomized and evaluated independently by three experienced observers on the basis of the following scoring: 1 - no signs of rejection, characterized by full grown pelt and original size of graft; 2 - signs of rejection, manifested by reduced number of hairs, cankerous appearance and reduction of graft size and/or dry scrap; 3 - rejection, characterized by reduction of graft to scar. For each graft the mean value of the individual scores given by the observers was used for quantification.

Immunoblotting. Joint capsule homogenates (50 – 200 µg) were separated by SDS-PAGE and immunoblotting was carried out using a rabbit polyclonal anti-MPO (myeloperoxidase) antibody (4162S, Cell Signaling). 50 µg lysate of $1.5*10^6$ neutrophils served as positive control. Anti-GAPDH rabbit antibody (MAB374, Sigma) was used as loading control and for normalization.

References

1. Hedrich HJ. Testing for Isohistogeneity (Skin Grafting). In: Adams M and Hedrich HJ, eds. *Genetic Monitoring of Inbred Strains of Rats: A Manual on Colony Management, Basic Monitoring Techniques, and Genetic Variants of the Laboratory Rat*. New York: John Wiley & Sons Inc; 1990:102-114.