Dysregulated transforming growth factor-beta mediates early bone marrow dysfunction in diabetes

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Short title: TGFB in bone marrow dysfunction in diabetes

Supplementary File:
Supplementary Figures and Tables: 22 figures and 11 tables.
**Supplementary Table S1: Media formulation for insulin-independent lipid accumulation in cells.**

| Component                                      | Catalogue #, Vendor                  | Diluent | [Stock: Final] | Control media | idADP + HG | idADP + LIN/OL | idADP + LIN/OL + HG |
|------------------------------------------------|--------------------------------------|---------|----------------|---------------|------------|----------------|---------------------|
| DMEM, low glucose, pyruvate                   | 11885084, Thermo Fisher              | -       | -              | +            | +          | +              | +                   |
| Fetal Bovine Serum, qualified, heat inactivated| 12484028, Thermo Fisher              | -       | 100%: 10%      | +            | +          | +              | +                   |
| Dexamethasone - water soluble                  | D2915, Sigma-Aldrich                 | EtOH    | 1 mM: 1 μM     | -            | +          | +              | +                   |
| Indomethacin                                   | I7378, Sigma-Aldrich                 | EtOH    | 50 mM: 50 μM   | -            | +          | +              | +                   |
| 3-Isobutyl-1-methylxanthine                    | I7018, Sigma-Aldrich                 | EtOH    | 50 mM: 0.5 mM  | -            | +          | +              | +                   |
| Dextrose, anhydrous                            | CABDH0230, VWR chemicals             | DMEM, low glucose | 1 M: 20 mM     | -            | -          | +              | -                   |
| Linoleic Acid-Oleic Acid-Albumin               | L9655, Sigma-Aldrich                 | -       | 100X: 2X       | -            | -          | -              | +                   |
| Antibiotic Antimycotic                         | 30-004-CI, Corning                   | -       | 100X: 1X       | +            | +          | +              | +                   |

*Abbreviations: ADP = adipogenesis induction media; idADP = insulin-deficient adipogenesis induction media; EtOH = ethanol; HG = high glucose (25 mmol/L); LIN/OL = linoleic acid-oleic acid.*
**Supplementary Table S2:** Gene ontology (GO) biological processes from target genes that are upregulated in bm-MPCs subjected to 1) adipogenic differentiation, and 2) adipogenic differentiation media with TGFβ1 and TAK1 inhibitor.

**Gene ontology (GO) biological processes enriched**

| Biological Process Term | p-value   |
|-------------------------|-----------|
| GO:0055114 oxidation-reduction process | 1.27E-05  |
| GO:0006635 fatty acid beta-oxidation | 8.83E-05  |
| GO:0006098 pentose-phosphate shunt | 1.70E-04  |
| GO:0015909 long-chain fatty acid transport | 2.25E-04  |
| GO:0006629 lipid metabolic process | 2.34E-04  |
| GO:0042493 response to drug | 0.001295  |
| GO:0006631 fatty acid metabolic process | 0.002047  |
| GO:0010886 positive regulation of cholesterol storage | 0.002167  |
| GO:0043524 negative regulation of neuron apoptotic process | 0.002535  |
| GO:0034383 low-density lipoprotein particle clearance | 0.002869  |
| GO:0055085 transmembrane transport | 0.003987  |
| GO:0042953 cellular oxidant detoxification | 0.006003  |
| GO:0042953 lipoprotein transport | 0.010259  |
| GO:0006646 phosphatidylethanolamine biosynthetic process | 0.010259  |
| GO:0050731 positive regulation of peptidyl-tyrosine phosphorylation | 0.010409  |
| GO:0010595 positive regulation of endothelial cell migration | 0.011979  |
| GO:0070542 response to fatty acid | 0.013109  |
| GO:0051968 positive regulation of synaptic transmission, glutamatergic | 0.016261  |
| GO:002904 respiratory electron transport chain | 0.017946  |
| GO:0035902 response to immobilization stress | 0.019702  |
| GO:0007173 epidermal growth factor receptor signaling pathway | 0.020296  |
| GO:0009051 pentose-phosphate shunt, oxidative branch | 0.020617  |
| GO:0042412 taurine biosynthetic process | 0.020617  |
| GO:0019322 pentose biosynthetic process | 0.020617  |
| GO:0019915 lipid storage | 0.025376  |
| GO:0033344 cholesterol efflux | 0.027397  |
| GO:0006656 phosphatidylethanolamine biosynthetic process | 0.027397  |
| GO:0032496 response to lipopolysaccharide | 0.028214  |
| GO:0006549 isoleucine metabolic process | 0.030767  |
| GO:0000255 allantoin metabolic process | 0.030767  |
| GO:0032868 response to insulin | 0.032316  |
| GO:0051881 regulation of mitochondrial membrane potential | 0.033832  |
| GO:0070374 positive regulation of ERK1 and ERK2 cascade | 0.035667  |
| GO:0006642 triglyceride mobilization | 0.040812  |
| GO:0046449 creatinine metabolic process | 0.040812  |
| GO:0006573 | valine metabolic process | 0.040812 |
|------------|--------------------------|----------|
| GO:0032869 | cellular response to insulin stimulus | 0.045823 |
| GO:0019221 | cytokine-mediated signaling pathway | 0.047437 |
Supplementary Table S3: Gene ontology (GO) biological processes from target genes that are downregulated in bm-MPCs subjected to 1) adipogenic differentiation, 2) adipogenic differentiation media with TGFB1 and TAK1 inhibitor.

| Biological Process Term                                               | p-value  |
|-----------------------------------------------------------------------|----------|
| GO:0007017 microtubule-based process                                   | 2.04E-04 |
| GO:0098609 cell-cell adhesion                                         | 3.86E-04 |
| GO:0045892 negative regulation of transcription, DNA-templated       | 0.002616 |
| GO:0090190 positive regulation of branching involved in ureteric bud morphogenesis | 0.010318 |
| GO:0001649 osteoblast differentiation                                  | 0.010401 |
| GO:0032922 circadian regulation of gene expression                    | 0.011277 |
| GO:1900275 negative regulation of phospholipase C activity           | 0.016251 |
| GO:0042384 cillum assembly                                            | 0.018749 |
| GO:0008652 cellular amino acid biosynthetic process                   | 0.018895 |
| GO:0007265 Ras protein signal transduction                             | 0.019547 |
| GO:0060271 cillum morphogenesis                                       | 0.025299 |
| GO:0045893 positive regulation of transcription, DNA-templated       | 0.025357 |
| GO:0001501 skeletal system development                                | 0.025888 |
| GO:0007155 cell adhesion                                              | 0.034578 |
| GO:0030177 positive regulation of Wnt signaling pathway               | 0.034745 |
| GO:0060071 Wnt signaling pathway, planar cell polarity pathway        | 0.039448 |
| GO:0055129 L-proline biosynthetic process                             | 0.040138 |
| GO:0007411 axon guidance                                              | 0.041325 |
| GO:0006561 proline biosynthetic process                               | 0.047971 |
**Supplementary Table S4: Gene sets enriched in bm-MPCs exposed to control and adipogenic differentiation media (ADP).**

| Condition | Gene Set                              | Size | NES        | p-value | FDR       |
|-----------|---------------------------------------|------|------------|---------|-----------|
| ADP       | Adipogenesis                          | 187  | 2.658365   | 0       | 0         |
|           | Fatty Acid Metabolism                 | 152  | 2.406275   | 0       | 0         |
|           | Xenobiotic Metabolism                 | 191  | 1.972802   | 0       | 0         |
|           | Cholesterol Homeostasis               | 69   | 1.875002   | 0.002268| 0.001414  |
|           | Oxidative Phosphorylation             | 176  | 1.788628   | 0       | 0.002573  |
|           | Peroxisome                           | 99   | 1.587389   | 0.002375| 0.014536  |
|           | Myc Targets V2                        | 51   | 1.538435   | 0.026846| 0.019612  |
|           | Bile Acid Metabolism                  | 110  | 1.517775   | 0.00907 | 0.021075  |
|           | Androgen Response                     | 92   | 1.491636   | 0.011494| 0.024066  |
|           | Apical Surface                        | 42   | 1.352561   | 0.090551| 0.069815  |
|           | Reactive Oxygen Species Pathway       | 45   | 1.237397   | 0.147752| 0.155475  |
|           | Pancreas Beta Cells                   | 39   | 1.207659   | 0.209607| 0.177652  |
|           | Angiogenesis                          | 34   | 1.15252    | 0.263393| 0.234508  |
|           | KRAS Signaling Dn                     | 190  | 1.075596   | 0.266366| 0.355486  |
|           | UV Response Up                        | 154  | 1.057003   | 0.310945| 0.369972  |
|           | Myc Targets V1                        | 174  | 1.040503   | 0.340376| 0.379482  |
|           | Spermatogenesis                       | 129  | 1.016069   | 0.412301| 0.409409  |
| Control   | Interferon Alpha Response             | 91   | -2.11756   | 0       | 0         |
|           | Interferon Gamma Response             | 192  | -2.11069   | 0       | 0         |
|           | Mitotic Spindle                       | 193  | -2.02258   | 0       | 2.75E-04  |
|           | Epithelial Mesenchymal Transition     | 192  | -2.05704   | 0       | 3.67E-04  |
|           | TNFa Signaling via NFKB               | 192  | -1.91684   | 0       | 6.50E-04  |
|           | Apical Junction                       | 187  | -1.86246   | 0       | 9.58E-04  |
|           | G2/M Checkpoint                       | 180  | -1.89573   | 0       | 0.001117  |
|           | Inflammatory Response                 | 195  | -1.77894   | 0       | 0.002031  |
|           | p53 Pathway                          | 185  | -1.76275   | 0       | 0.002376  |
|           | IL2 STAT5 Signaling                   | 190  | -1.72817   | 0       | 0.003334  |
|           | Protein Secretion                     | 92   | -1.68879   | 0.001825| 0.004221  |
|           | Apoptosis                             | 154  | -1.69173   | 0       | 0.004408  |
|           | Unfolded Protein Response             | 97   | -1.59861   | 0.003584| 0.008945  |
|           | Coagulation                           | 134  | -1.59909   | 0       | 0.006800  |
|           | Estrogen Response Early               | 188  | -1.60237   | 0.001689| 0.010118  |
|           | E2F Targets                           | 181  | -1.52685   | 0.003565| 0.016091  |
|           | UV Response Dn                       | 132  | -1.45121   | 0.017794| 0.032148  |
|           | Complement                            | 192  | -1.43812   | 0.005474| 0.034237  |
|           | KRAS Signaling Up                     | 192  | -1.42564   | 0.007156| 0.036671  |
|           | Allograft Rejection                   | 188  | -1.39622   | 0.028319| 0.045073  |
|           | Notch Signaling                       | 31   | -1.3424    | 0.102703| 0.06793   |
| Pathway                        | Score | P-value | FDR-value |
|-------------------------------|-------|---------|-----------|
| Hypoxia                       | 192   | -1.33269| 0.02131   |
| Estrogen Response Late        | 190   | -1.24963| 0.084577  |
| TGF Beta Signaling            | 53    | -1.21881| 0.164602  |
| PI3K AKt mTOR Signaling       | 100   | -1.13364| 0.207294  |
| IL6 JAK STAT3 Signaling       | 85    | -1.1351 | 0.242321  |
| Heme Metabolism               | 185   | -1.11822| 0.216028  |
| mTORC1 Signaling              | 192   | -1.07734| 0.289116  |
| Myogenesis                    | 190   | -1.05333| 0.328026  |
| Glycolysis                    | 192   | -1.02774| 0.389545  |
| Hedgehog Signaling            | 34    | -0.93503| 0.551595  |
| Wnt Beta Catenin Signaling    | 41    | -0.86948| 0.642857  |
| DNA Repair                    | 138   | -0.76945| 0.939338  |
Supplementary Table S5: Gene sets enriched in bm-MPCs exposed to adipogenic differentiation media (ADP) with or without TGFβ1.

| Condition | Gene Set                          | Size | NES      | p-value | FDR      |
|-----------|-----------------------------------|------|----------|---------|----------|
| ADP       | Interferon Alpha Response         | 91   | 2.191829 | 0       | 0        |
|           | Adipogenesis                      | 187  | 2.017069 | 0       | 0        |
|           | Xenobiotic Metabolism             | 191  | 1.895772 | 0       | 9.95E-04 |
|           | Fatty Acid Metabolism             | 152  | 1.871451 | 0       | 7.46E-04 |
|           | Bile Acid Metabolism              | 110  | 1.725169 | 0       | 0.002389 |
|           | Interferon Gamma Response         | 192  | 1.638922 | 0.003247| 0.007206 |
|           | Apical Surface                    | 42   | 1.406108 | 0.058076| 0.061861 |
|           | Cholesterol Homeostasis           | 69   | 1.393647 | 0.039076| 0.058844 |
|           | Complement                        | 192  | 1.36985  | 0       | 0.002389 |
|           | Peroxisome                        | 99   | 1.352695 | 0.055077| 0.069613 |
|           | Reactive Oxygen Species Pathway   | 45   | 1.271546 | 0.136937| 0.128324 |
|           | KRAS Signaling Dn                 | 190  | 1.237835 | 0.074919| 0.152356 |
|           | IL6 JAK STAT3 Signaling           | 85   | 1.105878 | 0.272727| 0.347811 |
|           | Myogenesis                        | 190  | 1.088949 | 0.271829| 0.357887 |
|           | Allograft Rejection               | 188  | 1.079157 | 0.288525| 0.354051 |
|           | Coagulation                       | 134  | 1.034265 | 0.390048| 0.426172 |
|           | Oxidative phosphorylation         | 176  | 0.96039  | 0.56042 | 0.57492  |
|           | Spermatogenesis                   | 129  | 0.90317  | 0.684474| 0.679257 |
| TGFβ1     | TGF Beta Signaling                | 53   | -2.12261 | 0       | 0        |
|           | Unfolded Protein Response         | 97   | -2.09296 | 0       | 0        |
|           | mTORC1 Signaling                  | 192  | -2.0696  | 0       | 0        |
|           | Epithelial Mesenchymal Transition | 192  | -2.03528 | 0       | 0        |
|           | Hypoxia                           | 192  | -2.01353 | 0       | 0        |
|           | Glycolysis                        | 192  | -1.94226 | 0       | 0        |
|           | TNFα Signaling via NFκB           | 192  | -1.90582 | 0       | 6.19E-04 |
|           | G2/M Checkpoint                   | 180  | -1.77283 | 0       | 0.001843 |
|           | p53 Pathway                       | 185  | -1.62084 | 0       | 0.009627 |
|           | E2F Targets                       | 181  | -1.57311 | 0       | 0.015059 |
|           | IL2 STAT5 Signaling               | 190  | -1.55387 | 0.002681| 0.017656 |
|           | Hedgehog Signaling                | 34   | -1.51943 | 0.033113| 0.022994 |
|           | Inflammatory Response             | 195  | -1.4207  | 0.010309| 0.047972 |
|           | Estrogen Response Early           | 188  | -1.35651 | 0.01061 | 0.078547 |
|           | Myc Targets V1                    | 174  | -1.33254 | 0.02267 | 0.083333 |
|           | UV Response Dn                    | 132  | -1.32667 | 0.031818| 0.087379 |
|           | Wnt Beta Catenin Signaling        | 41   | -1.30854 | 0.096552| 0.097154 |
|           | Protein Secretion                 | 92   | -1.30152 | 0.06988 | 0.096993 |
|           | UV Response Up                    | 154  | -1.2737  | 0.062035| 0.116662 |
|           | KRAS Signaling Up                 | 192  | -1.25186 | 0.046392| 0.131479 |
|           | Androgen Response                 | 92   | -1.24269 | 0.088095| 0.133734 |
|           | Apoptosis                         | 154  | -1.19927 | 0.099237| 0.17918  |
| Pathway                        | Value | p-value | q-value |
|-------------------------------|-------|---------|---------|
| PI3K AKT mTOR Signaling       | 100   | -1.18508| 0.16152 |
| Pancreas Beta Cells           | 39    | -1.17789| 0.210526| 0.19274 |
| Mitotic Spindle               | 193   | -1.17033| 0.097297| 0.194721|
| Angiogenesis                  | 34    | -1.06676| 0.339713| 0.376011|
| Apical Junction               | 187   | -1.04731| 0.315294| 0.408796|
| Estrogen Response Late        | 190   | -1.01412| 0.395161| 0.475929|
| Notch Signaling               | 31    | -0.96412| 0.486301| 0.595359|
| Myc Targets V2                | 51    | -0.92005| 0.586605| 0.696053|
| DNA Repair                    | 138   | -0.91215| 0.698565| 0.695802|
| Heme Metabolism               | 185   | -0.8993 | 0.716456| 0.705125|
Supplementary Table S6: Gene sets enriched in bm-MPCs exposed to adipogenic differentiation media and TGFB1 (TGFB1), with or without TAK1 inhibitor (TAK1i).

| Condition | Gene Set                          | Size | NES       | p-value | FDR         |
|-----------|-----------------------------------|------|-----------|---------|-------------|
| TGFB1     | G2/M Checkpoint                   | 180  | -1.95638  | 0       | 0           |
|           | Unfolded Protein Response         | 97   | -1.86891  | 0       | 4.47E-04    |
|           | E2F Targets                       | 181  | -1.65828  | 0       | 0.009373    |
|           | Hedgehog Signaling                | 34   | -1.58959  | 0.015119| 0.014036    |
|           | Myc Targets V1                    | 174  | -1.48678  | 0.004878| 0.027491    |
| TAK1i     | Adipogenesis                      | 187  | 2.356003  | 0       | 0           |
|           | Fatty Acid Metabolism             | 152  | 2.139756  | 0       | 0           |
|           | Xenobiotic Metabolism             | 191  | 2.105543  | 0       | 0           |
|           | Interferon Alpha Response         | 91   | 2.003802  | 0       | 5.90E-04    |
|           | Cholesterol Homeostasis           | 69   | 1.897892  | 0       | 0.001146    |
|           | Complement                        | 192  | 1.807903  | 0       | 0.001466    |
|           | p53 Pathway                       | 185  | 1.811716  | 0       | 0.001711    |
|           | Reactive Oxygen Species Pathway   | 45   | 1.750302  | 0.001815| 0.003332    |
|           | Oxidative Phosphorylation         | 176  | 1.693126  | 0       | 0.004221    |
|           | Bile Acid Metabolism              | 110  | 1.688197  | 0       | 0.004421    |
|           | Coagulation                       | 134  | 1.576186  | 0.001712| 0.012847    |
|           | Peroxisome                        | 99   | 1.518858  | 0.00495 | 0.019752    |
|           | Interferon Gamma Response         | 192  | 1.491824  | 0.006462| 0.023758    |
|           | Apoptosis                         | 154  | 1.371015  | 0.025682| 0.076556    |
|           | TNFα Signaling via NFKB           | 192  | 1.341559  | 0.027157| 0.08899     |
|           | Apical Junction                   | 187  | 1.347214  | 0.023256| 0.090629    |
|           | Myogenesis                        | 0.304618| 0.030303  | 0.099075| 0.79        |
|           | KRAS Signaling Up                 | 0.298357| 0.03882   | 0.110163| 0.837       |
|           | UV Response Dn                    | 0.312613| 0.080268  | 0.121307| 0.878       |
|           | Heme Metabolism                   | 0.296372| 0.0384    | 0.11931 | 0.883       |
|           | Estrogen Metabolism               | 0.281801| 0.094574  | 0.186286| 0.98        |
|           | IL6 JAK STAT3 Signaling           | 0.316327| 0.160584  | 0.211486| 0.988       |
|           | Estrogen Response Early           | 0.270939| 0.158516  | 0.259587| 0.998       |
|           | Hypoxia                           | 0.265547| 0.178571  | 0.247971| 0.999       |
|           | Apical Surface                    | 0.344719| 0.272381  | 0.257506| 0.999       |
|           | Inflammatory Response             | 0.267227| 0.189627  | 0.261144| 0.999       |
|           | DNA Repair                        | 0.272035| 0.193493  | 0.267666| 1           |
|           | UV Response Up                    | 0.236674| 0.470978  | 0.542542| 1           |
|           | KRAS Signaling Dn                 | 0.21025| 0.728988  | 0.783954| 1           |
|           | Androgen Response                 | 0.228339| 0.694946  | 0.809356| 1           |
|           | Angiogenesis                      | 0.258062| 0.753271  | 0.901269| 1           |
|           | Notch Signaling                   | 0.252517| 0.784133  | 0.942504| 1           |
|           | Protein Secretion                 | 0.146962| 1         | 0.998405| 1           |

Supplementary Figures and Tables
| Pathway                                   | Pathway ID | Log2 Fold Change | Log10 p-value | Log10 FDR  |
|-------------------------------------------|------------|------------------|---------------|------------|
| Epithelial Mesenchymal Transition         | 192        | -1.50045         | 0             | 0.028229   |
| Mitotic Spindle                           | 193        | -1.29085         | 0.028947      | 0.119671   |
| Myc Targets V2                            | 51         | -1.27503         | 0.121352      |            |
| mTORC1 Signaling                          | 192        | -1.25921         | 0.040284      | 0.125785   |
| Spermatogenesis                           | 129        | -1.29553         | 0.13208       |            |
| Pancreas Beta Cells                       | 39         | -1.18443         | 0.206505      |            |
| Allograft Rejection                       | 188        | -1.16725         | 0.215174      |            |
| IL2 STAT5 Signaling                       | 190        | -1.11181         | 0.295312      |            |
| TGF Beta Signaling                        | 53         | -1.05677         | 0.369251      |            |
| PI3K AKT mTOR Signaling                   | 100        | -1.05738         | 0.393633      |            |
| Glycolysis                                | 192        | -1.0338          | 0.395607      |            |
| Wnt Beta Catenin Signaling                | 41         | -0.92121         | 0.654772      |            |
Supplementary Table S7: qPCR primers for mouse genes.

| Gene  | Gene description                                      | Chemistry | Source (Cat#, Reference) |
|-------|-------------------------------------------------------|-----------|--------------------------|
| Actb  | Actin, beta                                           | SYBR-green| Qiagen (QT00095242)     |
| Actb  | Actin, beta                                           | Taqman    | Thermo Fisher (Mm02619580_g1) |
| Adipoq| Adiponectin                                           | SYBR-green| Thermo Fisher [1]         |
| Angpt1| Angiopoietin 1                                        | Taqman    | Thermo Fisher (Mm00456503_m1) |
| Atp5f1| ATP synthase, H + transporting, mitochondrial F0 complex, subunit B1 | SYBR-green| Thermo Fisher [2]         |
| Bmp4  | Bone morphogenetic protein 4                         | Taqman    | Thermo Fisher (Mm00432087_m1) |
| Cebpa | CCAAT Enhancer Binding Protein Alpha                  | SYBR-green| Thermo Fisher [1]         |
| Cnnb1 | Catenin (cadherin associated), beta 1                 | Taqman    | Thermo Fisher (Mm00483039_m1) |
| Cxcl12| C-X-C Motif Chemokine Ligand 12                       | SYBR-green| Thermo Fisher [1]         |
| Cxcl12| Chemokine (C-X-C motif) ligand 12                     | Taqman    | Thermo Fisher (Mm00445553_m1) |
| Cxcr4 | Chemokine (C-X-C motif) receptor 4                    | Taqman    | Thermo Fisher (Mm01292123_m1) |
| Fabp4 | Fatty acid binding protein 4, adipocyte               | SYBR-green| Qiagen (QT00091532)       |
| Gapdh | Glyceraldehyde-3-phosphate dehydrogenase              | Taqman    | Thermo Fisher (Mm99999915_g1) |
| Icam1 | Intercellular adhesion molecule 1 (also known as CD54)| SYBR-green| QT00155078               |
| Kit   | Kit oncogene                                           | Taqman    | Thermo Fisher (Mm00445212_m1) |
| Kitl  | Kit ligand                                            | Taqman    | Thermo Fisher (Mm00442972_m1) |
| Lepr  | Leptin receptor                                       | SYBR-green| Qiagen (QT00154133)       |
| Lpl   | Lipoprotein lipase                                     | SYBR-green| Thermo Fisher [1]         |
| Ly6a  | Lymphocyte antigen 6 complex; also known as Sc a1 (stem cell antigen-1) | Taqman    | Thermo Fisher (Mm00726565_s1) |
| Map3k7| mitogen-activated protein kinase kinase 7 (TAK1)      | Taqman    | Thermo Fisher (Mm00554514_m1) |
| Nanog | Nanog homeobox                                        | Taqman    | Thermo Fisher (Mm02019550_s1) |
| Pgk1  | Phosphoglycerate kinase 1                             | SYBR-green| Thermo Fisher [23]        |
| Pou5f1| POU domain, class 5, transcription factor 1; also known as Oct4 (Octamer-binding protein 4) | Taqman    | Thermo Fisher (Mm03053917_g1) |
| Pparg | Peroxisome proliferator activated receptor gamma       | SYBR-green| Qiagen (QT00100296)       |
| Pparg | Peroxisome proliferator activated receptor gamma       | SYBR-green| Qiagen (QT00100296)       |
| Ppargc| Peroxisome proliferator activated receptor gamma       |          |                          |
| Ppargc| Peroxisome proliferator activated receptor gamma       |          |                          |
| Ppargc| Peroxisome proliferator activated receptor gamma       |          |                          |
| Runx2 | Runt related transcription factor 2                   | Taqman    | Thermo Fisher (Mm01293577_m1) |
| Smad2 | SMAD family member 2                                  | Taqman    | Thermo Fisher (Mm00501584_m1) |
| Smad3 | SMAD family member 3                                  | Taqman    | Thermo Fisher (Mm01170760_m1) |
| Gene    | Description                                                                 | qPCR Method | Taqman (rd)  |
|---------|------------------------------------------------------------------------------|-------------|--------------|
| Smad6   | SMAD family member 6                                                         | Taqman      | Thermo Fisher (Mm00484738_m1) |
| Sox2    | SRY (sex determining region Y)-box 2                                         | Taqman      | Thermo Fisher (Mm03053810_s1) |
| Sp7     | Sp7 transcription factor                                                     | SYBR-green  | Qiagen (QT00293181) |
| Sp7     | Sp7 transcription factor                                                     | Taqman      | Thermo Fisher (Mm04933803_m1) |
| Tgfb1   | Transforming growth factor, beta 1                                           | SYBR-green  | Qiagen (QT00145250) |
| Tgfb1   | Transforming growth factor, beta 1                                           | Taqman      | Thermo Fisher (Mm01178820_m1) |
| Tgfb1   | Transforming growth factor, beta receptor 1                                  | SYBR-green  | Qiagen (QT00135828) |
| Tgfb1   | Transforming growth factor, beta receptor 1                                  | Taqman      | Thermo Fisher (Mm00436964_m1) |
| Tgfb2   | Transforming growth factor, beta receptor 2                                  | SYBR-green  | Qiagen (QT00135646) |
| Tgfb2   | Transforming growth factor, beta receptor 2                                  | Taqman      | Thermo Fisher (Mm00436977_m1) |
| Vcam1   | Vascular cell adhesion protein 1                                             | SYBR-green  | Qiagen (QT00128793) |
| Wnt11   | wingless-type MMTV integration site family, member 11                         | Taqman      | Thermo Fisher (Mm00437328_m1) |
Supplementary Table S8: qPCR primers for human genes.

| Gene   | Gene description                                      | Chemistry   | Qiagen Cat#                  |
|--------|-------------------------------------------------------|-------------|------------------------------|
| ACTB   | Actin, beta (housekeeping gene)                       | SYBR-green  | PPH00073G; QT01680476        |
| ACTB   | Actin, beta (housekeeping gene)                       | Taqman      | Hs01060665_g1                |
| ACSL1  | Acyl-CoA synthetase long chain family member 1        | Taqman      | Hs00960561_m1                |
| B2M    | Beta-2-microglobulin (housekeeping gene)             | SYBR-green  | PPH01094E                    |
| BGLAP  | Bone gamma-carboxyglutamate protein                   | SYBR-green  | PPH00073G; QT01680476        |
| BMPR1A | Bone morphogenetic protein receptor, type IA          | SYBR-green  | PPH01929C; QT00085358        |
| BMPR1B | Bone morphogenetic protein receptor, type IB          | SYBR-green  | PPH01952C; QT00084469        |
| BMPR2  | Bone morphogenetic protein receptor, type II          | SYBR-green  | PPH00401B; QT00226065        |
| CCND1  | Catenin (cadherin-associated), beta 1                 | Taqman      | Hs00354519_m1                |
| CTNNB1 | Catenin (cadherin-associated), beta 1                 | SYBR-green  | PPH00643F; QT00077882        |
| FGF1   | Fibroblast growth factor 1 (acidic)                  | SYBR-green  | PPH00067F                    |
| FGF2   | Fibroblast growth factor 2 (basic)                   | SYBR-green  | PPH00257C                    |
| FGFR1  | Fibroblast growth factor receptor 1                  | SYBR-green  | PPH00372F                    |
| FGFR2  | Fibroblast growth factor receptor 2                  | SYBR-green  | PPH00391F                    |
| GAPDH  | Glyceraldehyde-3-phosphate dehydrogenase (housekeeping gene) | SYBR-green  | PPH00150F; QT00079247        |
| HPRT1  | Hypoxanthine phosphoribosyltransferase 1 (housekeeping gene) | SYBR-green  | PPH01018C                    |
| IGF1   | Insulin-like growth factor 1 (somatomedin C)         | SYBR-green  | PPH00167C                    |
| IGF1R  | Insulin-like growth factor 1 receptor                | SYBR-green  | PPH00350F                    |
| IGF2   | Insulin-like growth factor 2 (somatomedin A)         | SYBR-green  | PPH00168B                    |
| LEF1   | Lymphoid enhancer-binding factor 1                   | SYBR-green  | PPH02778C; QT00021133        |
| PPARG2 | Peroxisome proliferator-activated receptor gamma, isoform 2 | SYBR-green  | QT00029841                   |
| RPLP0  | Ribosomal protein, large, P0 (housekeeping gene)     | SYBR-green  | PPH21138F; QT00075012        |
| RTC    | Reverse Transcription Control                        | SYBR-green  | PPX63340A                    |
| RUNX2  | Runt-related transcription factor 2                  | SYBR-green  | PPH01897C; QT00020517        |
| SLC27A1| Solute carrier family 27 member 1 (also known as fatty acid transport protein 1, FATP1) | Taqman      | Hs01587911_m1                |
| SMAD1  | SMAD family member 1                                 | SYBR-green  | PPH01174A                    |
| SMAD2  | SMAD family member 2                                 | SYBR-green  | PPH01949F                    |
| SMAD3  | SMAD family member 3                                 | SYBR-green  | PPH01921C                    |
| SMAD4  | SMAD family member 4                                 | SYBR-green  | PPH00134C                    |
| SMAD5  | SMAD family member 5                                 | SYBR-green  | PPH01940C                    |
| SP7    | Sp7 transcription factor                             | SYBR-green  | PPH00705A; QT00213514        |
| TCF7   | Transcription factor 7 (T-cell specific, HMG-box)    | SYBR-green  | PPH02757B; QT00095410        |
| Gene      | Description                                           | Primer Type | Accession Numbers          |
|-----------|-------------------------------------------------------|-------------|----------------------------|
| TCF7L1    | Transcription factor 7-like 1 (T-cell specific, HMG-box) | SYBR-green  | PPH02745B; QT00091735      |
| TCF7L2    | Transcription factor 7-like 2 (T-cell specific, HMG-box) | SYBR-green  | PPH06890C; QT00071120      |
| TGFB1     | Transforming growth factor, beta 1                    | SYBR-green  | PPH00508A; QT00000728      |
| TGFB2     | Transforming growth factor, beta 2                    | SYBR-green  | PPH00524B                  |
| TGFB3     | Transforming growth factor, beta 3                    | SYBR-green  | PPH00531F                  |
| TGFBR1    | Transforming growth factor, beta receptor 1           | SYBR-green  | PPH00237C; QT00083412      |
| TGFBR2    | Transforming growth factor, beta receptor II (70/80kDa) | SYBR-green  | PPH00339C                  |
| WISP1     | WNT1 inducible signaling pathway protein 1            | SYBR-green  | QT00079492                 |
**Supplementary Table S9: Antibodies and conditions for mouse bone tissue staining.**

| Target | Alternative names | Clone | Source           | Catalogue number | Host Species | Antigen Retrieval Buffer | Figure # |
|--------|-------------------|-------|------------------|------------------|--------------|--------------------------|----------|
| CD45   | Ly5, LCA          | Poly  | Thermo Fisher    | 20103-1-AP       | Rabbit       | TRIS/EDTA                | S11      |
| CXCL12 | SDF-1             | Poly  | Thermo Fisher    | 14-7992-81       | Rabbit       | TRIS/EDTA                | S16      |
| INS    |                   | Poly  | Thermo Fisher    | 15848-1-AP       | Rabbit       | Citrate/EDTA             | S2       |
| LEPR   |                   | Poly  | R&D Systems      | AF497            | Mouse        | Citrate/EDTA             | S18      |
| PLIN1  |                   | Poly  | Thermo Fisher    | PA5-55046        | Rabbit       | TRIS/EDTA                | 1, S6    |
| TGFB1  |                   | Poly  | Proteintech      | 21898-1-AP       | Rabbit       | TRIS/EDTA                | 3        |
| SCA1   | Ly6A              | Mono  | Abcam            | ab109211         | Rabbit       | Citrate/EDTA             | 2        |
| SOX2   |                   | Poly  | Thermo Fisher    | 48-1400          | Rabbit       | TRIS/EDTA                | 2        |
**Supplementary Table S10: Exposure times of various test agents in cell culture studies.**

| Illustration # | Test agents                                                                 | Exposure duration |
|----------------|------------------------------------------------------------------------------|-------------------|
| Figure 5       | Adipogenesis inducing media (ADP); recombinant human transforming growth factor beta 1 (TGFB1); 25 mM glucose (HG) | 72 hours          |
| Figure 6       | Adipogenesis inducing media (ADP); recombinant human transforming growth factor beta 1 (TGFB1); signaling inhibitors (ALK5, TAK1, JNK, SMAD3, P38, ERK, PKC, PI3K) | 72 hours          |
| Figure 7; Table S7-S11 | Adipogenesis inducing media (ADP); recombinant human transforming growth factor beta 1 (TGFB1); TAK1 inhibitor | 48 hours          |
| Figure S10     | Insulin-deficient adipogenesis induction media (idADP); Linoleic-Oleic acid (LIN/OL); 25 mM glucose (HG) | 7 days            |
| Figure S20     | 25 mM glucose (HG)                                                          | 21 days           |
| Figure S21     | Adipogenesis inducing media (ADP); recombinant human transforming growth factor beta 1 (TGFB1); 25 mM glucose (HG); TALK1 inhibitor | 72 hours          |
| Figure S22     | Osteogenesis inducing media (OST); recombinant human transforming growth factor beta 1 (TGFB1) | 9 days            |
**Supplementary Table S11: Pharmacological inhibitors used in cell culture studies.**

| Reagent               | Target protein | Acronym | Concentrations tested | Source (Catalogue #)       |
|-----------------------|----------------|---------|------------------------|----------------------------|
| GW 788388             | ALK5           | ALK5i   | 1, 10, 50 µmol/L       | Tocris Bioscience (3264)   |
| Chelerythrine Chloride| PKC            | PKCi    | 0.1, 1, 5 µmol/L       | Cayman Chemical (11314)    |
| SB203580              | p38 MAPK       | p38i    | 1, 10, 50 µmol/L       | Millipore (559395)         |
| (5Z)-7-Oxozaenol      | TAK1           | TAK1i   | 1, 10, 50 µmol/L       | Cayman Chemical (17459)    |
| JNK inhibitor XVI     | JNK            | JNKi    | 1, 10, 50 µmol/L       | Cayman Chemical (18096)    |
| SIS3                  | SMAD3          | SMAD3i  | 1, 10, 50 µmol/L       | Cayman Chemical (15945)    |
| PI 828                | PI3K           | PI3Ki   | 1, 10, 50 µmol/L       | Tocris Bioscience (2814)   |
| PD98059               | MEK1,2         | MEKi    | 1, 10, 50 µmol/L       | STEMCELL Technologies (72172) |
Supplementary Figure S1: *Streptozotocin-induced diabetes causes impaired weight gain in mice at 1 month.*

Diabetes was induced in C57BL/6 mice with daily intraperitoneal injections of streptozotocin (STZ; 50 mg/kg) for 5 consecutive days. Non-diabetic control mice received an equal volume of citrate buffer. Blood glucose levels were checked 1 week after the last STZ injection to confirm hyperglycemia. (a) The body weight difference was calculated 1 week after the last STZ injection. [Mean ± SD; n = 7; each data point represents a mouse; two-tailed student’s t-test: * p<0.05]. (b) Non-fasting blood glucose levels in mice measured 1 week after the last STZ injection [Mean ± SD; n = 5-7; each data point represents a mouse; two-tailed student’s t-test: * p<0.05]. (c) Body weight difference at the conclusion of the study (d29) [Mean ± SD; n = 7; each data point represents a mouse; two-tailed student’s t-test: * p<0.05]. (d) Non-fasting blood glucose levels measured at the conclusion of the study (d28) [Mean ± SD; n = 7; each data point represents a mouse; two-tailed student’s t-test: * p<0.05]. (e) Cumulative average body weight
difference from d-11 to d29 comparing non-diabetic Control and STZ mice [Mean ± SD; n = 7; Two-way ANOVA followed by Bonferroni post hoc analysis: * p<0.05].
**Supplementary Figure S2: Diminished pancreatic islets in streptozotocin-induced diabetic mice at 1 month.**

Pancreata were harvested from C57BL/6 male mice, 1 month after administration of streptozotocin (STZ; 50 mg/kg) or citrate buffer control (Control). (a) Representative images showing H&E-stained sections (upper panel) and insulin immunoreactivity (lower panel). Insulin reactivity was detected by chromogen staining (arrow in STZ group indicating positive reactivity in a diminished islet) [scale bar = 200 μm for H&E and 100 μm for insulin staining]. (b) The number of pancreatic islets (insulin-positive islets; manual count) per pancreatic area (determined by QuPath) is shown [Mean ± SD; n = 4 control and 5 STZ; each data point represents a mouse; two-tailed student's t-test: * p<0.05].
Supplementary Figure S3: Histological analyses of STZ-induced diabetic mice at 1 month.

Tissues were harvested from C57BL/6 male mice, 1 month after administration of streptozotocin (STZ; 50 mg/kg) or citrate buffer control (Control). (a) Representative H&E-stained images of heart, lung, kidney, and liver tissue of mice [scale bar = 100 μm]. (b) Periodic Acid-Schiff (PAS)-stained images of retina, heart, and kidney tissues [scale bar = 100 μm]. (c) Picro-Sirius red-stained tissues of mice [scale bar = 100 μm]. (d-f) Quantitative assessment of Picro-Sirius red-stained area of the heart (d), lung (e), and kidney (f). Stained areas were determined with...
ImageJ [Mean ± SD; n = 4 controls and 4 STZ in panels d and e, 3 controls and 4 STZ in panel f; each data point represents a mouse; two-tailed student’s t-test: * p<0.05].
Supplementary Figure S4: Reduced inner nuclear layer thickness of the retina in mice after 1 month of streptozotocin-induced diabetes.

Eyes were harvested from C57BL/6 male mice, 1 month after administration of streptozotocin (STZ; 50 mg/kg) or citrate buffer control (Control). (a) Representative H&E-stained sections of the retina in C57BL/6 mice [scale bar = 50 μm]. (b-i) The thickness of the retinal layer was measured as a percentage of the neuronal retina thickness in the control and diabetic mouse [Mean ± SD; n = 6-8; each data point represents a mouse; two-tailed student’s t-test: * p<0.05]. Abbreviations: NFL, nerve fiber layer; GCL, ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; PL, photoreceptor layer; and GCC, ganglion cell complex.
Supplementary Figure S5: Cellular hyperplasia in white adipose tissue of streptozotocin-induced diabetic mice at 1 month.

Epididymal fat was harvested from C57BL/6 male mice, 1 month after administration of streptozotocin (STZ; 50 mg/kg) or citrate buffer control (Control). (a) Representative H&E-
stained sections [scale bar = 50 μm]. Inserts showing higher magnification. (b) The number of nuclei per tissue area was measured by QuPath [Mean ± SD; n = 6 control and 7 STZ; each data point represents a mouse; two-tailed student’s t-test: * p<0.05]. (c) Frequency of the area of the individual adipocytes of different sizes found in epididymal adipose tissue. Frequency was determined by Adiposoft [Mean ± SD; n = 6 control and 7 STZ; two-way ANOVA followed by Bonferroni post hoc analysis: * p<0.05]. (d-h) mRNA levels of Pparg (d), Cebpa (e), Fabp4 (f), Lpl (g), and Adipoq (h) in epididymal adipose tissue [Data normalized to Actb; Mean ± SD; n = 6 control and 7 STZ; each data point represents a mouse; two-tailed student’s t-test: * p<0.05].
Supplementary Figure S6: Increased lipid accumulation in the liver of streptozotocin-induced diabetic mice at 1 month.

Liver tissues were harvested from C57BL/6 male mice, 1 month after administration of streptozotocin (STZ; 50 mg/kg) or citrate buffer control (Control). (a) Representative images of the liver showing periodic Acid-Schiff (PAS) staining performed with or without the combination of diastase (α-amylase) [scale bar = 50 μm]. Inserts showing higher magnification. (b) Immunofluorescence staining of liver tissues for perilipin-1 (PLIN1; green). Sections were counterstained with DAPI (blue) [scale bar = 50 μm]. Inserts showing higher magnification.
Supplementary Figure S7: Changes in femur and tibia length after 1 month of diabetes.

(a,b) Lengths of the tibia (a) and femur (b) were measured by QuPath in control and streptozotocin (STZ)-induced diabetic mice after 1 month [Mean ± SD; n=7 control and 6 STZ in panel a, 4 control and 5 STZ in panel b; each data point represents a mouse; two-tailed student’s t-test: * p<0.05].
TGFB in bone marrow dysfunction in diabetes

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Supplementary Figures and Tables

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Supplementary Figure S8: *Quality control of the bone marrow flush method.*

Representative hematoxylin and eosin (H&E)-stained sections of mouse tibia and femur after marrow flush (‘flushed’) or without flush (‘whole’). Figure showing areas of the bone shaft (a) and distal ends (b) [scale bar = 200 μm]. (c) Whole femur and tibia after flush [scale bar = 1000 μm].
Supplementary Figure S9: Expression of adipogenesis-associated genes shows no difference in the bone marrow after 1 month of streptozotocin-induced diabetes.

Tibiae (a-c) and femurs (d-f) of non-diabetic control and streptozotocin (STZ)-induced diabetic mice, 1 month after the onset of diabetes, were flushed to isolate marrow cells for mRNA analyses. Levels of adipogenesis-associated genes were measured [For panels (a,d), data normalized to Actb and Gapdh; for panels (b,c,e,f), data normalized to Actb, Atp5f1, and Pgk1; Mean ± SD; n = 5 control and 7 STZ in panel a, 6 control and 6 STZ in panel b, 5 control and 7 STZ in panel c, 6 control and 7 STZ in panel d, 6 control and 6 STZ in panels e and f; each data point represents a mouse; two-tailed student’s t-test: * p<0.05].
Supplementary Figure S10: Insulin-independent lipid accumulation in bone marrow-derived mesenchymal progenitor cells.

Human bone marrow-derived progenitor cells (bm-MPCs) were exposed to an insulin-deficient adipogenesis induction media (idADP; Table S5) for 7 days. idADP was supplemented with either 25 mM glucose (high glucose; HG), linoleic acid-oleic acid mixture (LIN/OL), or a combination of HG and LIN/OL. Upper panel showing phase contrast images of cells [arrows point to lipid droplets in cells exposed to idADP]. Lower panel showing cells stained with LipidTOX (green) to detect intracellular lipid accumulation. Images are representative of 4 experimental replicates [scale bar = 50 μm]. Inserts showing high-power images.
Supplementary Figure S11: Hematopoietic area in the tibia of streptozotocin-induced diabetic mice at 1 month is not altered.

(a) Immunofluorescence staining of the tibiae of control and diabetic (STZ; 1 month) mice for CD45 (green). Sections were counterstained with DAPI (blue) [scale bar = 50 μm]. Inserts showing higher magnification.  
(b) Quantification of CD45 intensity per area, as determined by ImageJ [Mean ± SD; n = 7 control and 7 STZ; each data point represents an independent sample; two-tailed student’s t-test: * p<0.05].  
(c) Cd45 (Ptprc) mRNA levels in tibia flush samples [Data normalized to Actb and Gapdh; Mean ± SD; n = 6 control and 7 STZ; each data point represents a mouse; two-tailed student’s t-test: * p<0.05].  
(d) Nucleated area per bone area measured using MarrowQuant [Mean ± SD; n = 6 control and 7 STZ; each data point represents a mouse; two-tailed student’s t-test: * p<0.05].
Supplementary Figure S12: Localization of Tgfβ1 mRNA in the tibia of non-diabetic and streptozotocin-induced diabetic mice.

Representative images of tibiae of control and diabetic (STZ; 1 month) mice for Tgfβ1 (red). Sections were counterstained with hematoxylin (blue). Bacillus subtilis dihydrodipicolinate reductase (dapb) probe was used as a negative control [scale bar = 100 μm for low power images on left, 50 μm for high power images on right]. Inserts showing higher magnification. Asterisks highlighting select megakaryocytes.
Supplementary Figure S13: Fluorescence images of Tgfb1 in situ hybridization.

Representative images of tibiae of control and diabetic (STZ; 1 month) mice for Tgfb1 (red) [scale bar = 50 μm]. Bacillus subtilis dihydrodipicolinate reductase (dapb) probe was used as a negative control. Probes were detected RNAscope 2.5 HD Assay Red (chromogen-based; Red). Fluorescence images were taken using TRITC filter. RNAscope 2.5 Red Reagent is naturally fluorescent around 570 nm (TRITC/CY3). Inserts showing higher magnification. Asterisks highlighting select megakaryocytes. Second-level inserts (in green) show that most bone marrow cells express Tgfb1.
Supplementary Figure S14: Impaired weight gain and hyperglycemia in diabetic mice at 2 months.

C57BL/6 male mice received streptozotocin (STZ; 50 mg/kg) or citrate buffer (non-diabetic controls). (a) Body weights of mice at 2 months of follow-up [Mean ± SD; n = 6; each data point represents a mouse; two-tailed student’s t-test: * p<0.05]. (b) Non-fasting blood glucose levels in mice, 2 months after the onset of diabetes [Mean ± SD; n = 6; each data point represents a mouse; two-tailed student’s t-test: * p<0.05].
Supplementary Figure S15: *Picro-Sirius Red staining of tissues harvested from diabetic mice at 2 months.*

Tissues were harvested from C57BL/6 male mice, 2 months after the administration of streptozotocin (STZ; 50 mg/kg) or citrate buffer control (Control). Figure showing Picro-Sirius red-stained tissues of mice [n = 2; scale bar = 100 μm].
Supplementary Figure S16: Diabetes reduces stem cell niche factors in the bone marrow at 1 month.

(a-h) mRNA levels of Cxcl12, Cxcr4, Kitl, and Kit in the tibiae (a-d) and femurs (e-h) of control or diabetic (STZ) mice. Analyses were performed 1 month after the onset of diabetes [For panels (a-d) and (f-h), data normalized to Actb and Gapdh; for panel (e), data normalized to Actb, Atp5f1, and Pgk1; Mean ± SD; n = 5 control and 6 STZ in panel a, 5 control and 7 STZ in panel b, 5 control and 6 STZ in panel c, 6 control and 7 STZ in panel d, 6 control and 7 STZ in panel e,
6 control and 6 STZ in panel f, 6 control and 7 STZ in panels g and h; each data point represents a mouse; two-tailed student’s t-test: * p<0.05.  (i) Immunostaining of mouse tibia marrow for CXCL12 (green) after 1 month of STZ-induced diabetes. Sections were counterstained with DAPI (blue) [scale bar = 100 μm]. Inserts showing higher magnification.  (j) Quantification of CXCL12 intensity per area, as determined by ImageJ [Mean ± SD; n = 4; each data point represents an independent sample; two-tailed student’s t-test: * p<0.05].  (k, l) mRNA levels of Cxcl12 were detected in the tibiae (k) and femurs (l) of control or diabetic (STZ) mice after 2 months of diabetes onset [Data normalized to Actb and Gapdh; Mean ± SD; n = 4 control and 4 STZ in panel k, 3 control and 3 STZ in panel l; each data point represents a mouse; two-tailed student’s t-test: * p<0.05].
Supplementary Figure S17: *Streptozotocin-induced diabetic mice show no changes in mRNA levels of other known stem cell niche factors.*

(a-c) mRNA levels of *Icam1*, *Vcam1*, and *Angpt1* in the tibia flush samples from control and streptozotocin (STZ)-induced diabetic mice after 1 month of diabetes onset [For panels a and b, data normalized to *Actb*, *Atp5f1*, and *Pgk1*; for panel c, data normalized to *Actb* and *Gapdh*; Mean ± SD; n = 6 control and 6 STZ in panel a, 3 control and 3 STZ in panel b, 6 control and 7 STZ in panel c; two-tailed student’s t-test: * p<0.05].
Supplementary Figure S18: *Streptozotocin-induced diabetic mice show no changes in LEPR-expressing cells at 1 month.*

(a) Representative immunofluorescence staining of LEPR (green) in the shaft of tibia. Sections were counterstained with DAPI (blue) [scale bar = 50 μm]. (b) Quantification of LEPR staining intensity per area, as determined by *ImageJ* [Mean ± SD; n = 6 control and 5 STZ in panel b, 6 control and 7 STZ in panel c; two-tailed student’s t-test: *p*<0.05]. (c) *Lepr* mRNA levels in the tibia flush samples from control and diabetic (STZ) mice, after 1 month of diabetes onset [Data normalized to *Actb, Atp5f1*, and *Pgk1*; Mean ± SD; n = 5-6; each data point represents a mouse; two-tailed student’s t-test: *p*<0.05].
Supplementary Figure S19: Wnt signaling pathway genes are unaltered in the marrow of mice after 1 month of diabetes.

mRNA levels of Ctnnb1 (catenin beta-1) and Wnt11 (non-canonical Wnt ligand) were detected in the tibiae (a,b) and femurs (c,d) of control or diabetic (STZ) mice after 1 month of diabetes onset [Data normalized to Actb and Gapdh; Mean ± SD; n = 6 control and 6 STZ in panel a, 3 control and 5 STZ in panel b, 6 control and 6 STZ in panel c, 5 control and 5 STZ in panel d; each data point represents a mouse; two-tailed student’s t-test: * p<0.05].
Human bone marrow-derived progenitor cells were cultured in high glucose (25 mmol/L) for 21 days. Control cells were cultured in media containing 5 mmol/L glucose. Media was changed every other day. mRNA levels of TGFB ligands (a), TGFB receptors (b), BMP receptors (c), SMADs (d), fibroblast growth factor (FGF) signaling pathway (e), and insulin-like growth factor (IGF) signaling pathway (f) were measured. Red dashed line showing mean levels in control cells [Data normalized to ACTB, B2M, GAPDH, HPRT1, and RPLP0; n = 2; each data point represents an independent sample; two-tailed student’s t-test: * p<0.05].
Supplementary Figure S21: TGFβ1 modulates canonical Wnt regulators.

(a-e) Bone marrow-derived progenitor cells (bm-MPCs) were cultured in adipogenic media (ADP) with or without TGFβ1 (10 ng/mL) for 72 hours. mRNA levels of canonical Wnt pathway in bm-MPCs were measured [Data normalized to ACTB, B2M, GAPDH, HPRT1, and RPLP0; n = 2; each data point represents an independent sample; ANOVA followed by Bonferroni post hoc analysis: * p<0.05 compared with control, † p<0.05 compared with ADP].

(f) Immunofluorescence staining of bm-MPCs for TLE1 (green). Cells were cultured in ADP media, TGFβ1 (10 ng/mL), or ADP media containing TGFβ1 (10 ng/mL) for 72 hours. Cells were counterstained with DAPI (blue) [scale bar = 50 μm].

(g, h) mRNA levels of Wnt signaling response genes in bm-MPCs cultured in TGFβ1 (10 ng/mL) for 72 hours [Data normalized to ACTB; Mean ± SD; n = 3; each data point represents an independent sample; two-tailed student’s t-test: * p<0.05 compared with control].

(i, j) bm-MPCs were cultured in adipogenic media (ADP) with TGFβ1 (10 ng/mL) and (5Z)-7-Oxozeaenol (TAK1 inhibitor; TAK1i; 1 and 10 μmol/L) for 72 hours. mRNA levels of PPARγ2 (i) and WISP1 (j) were determined [Data normalized to ACTB; n = 4; each data point represents an independent sample; ANOVA]
followed by Bonferroni post hoc analysis: * p<0.05 compared with ADP, † p<0.05 compared with ADP+TGFβ1].
Supplementary Figure S22: TGFB1 inhibits late osteogenic differentiation factor in bm-MPCs and may hinder mineralization.

Bone marrow-derived mesenchymal progenitor cells (bm-MPCs) were cultured in osteogenic media (OST) with or without TGFB1 (10 ng/mL) for 9 days. (a-c) mRNA levels of osteogenesis-associated transcription factors [Data normalized to ACTB, GAPDH, and RPLP0; Mean ± SD; n = 4 OST and 5 OST+TGFB1 in panels a and b, 5 OST and 5 OST+TGFB1 in panel c; each data point represents an independent sample; two-tailed student’s t-test: * p<0.05]. (d) bm-MPCs cultured in osteogenic media (OST) with TGFB1 (10 ng/mL) for 9 days were assessed for mineralized matrix and calcium deposition by Alizarin Red S staining [representative of n=3; n is an independent sample; scale bar = 100 μm]. (e) Quantification of the Alizarin Red staining S was performed by measuring absorbance at 405 nm [Data normalized to control conditions; Mean ± SD; n = 3; each data point represents an independent sample; two-tailed student’s t-test: * p<0.05 compared with OST].
Supplemental References

Supplemental references relate to primer sequences for qPCR (Supplementary Table S7).

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