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β-Cell Regeneration Mediated by Human Bone Marrow Mesenchymal Stem Cells

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Stefanie Nickl

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Background
Mesenchymal Stem Cells

- First isolation from bone marrow 30 ys ago
- Isolation from: spleen, heart, skeletal muscle, synovium, amniotic fluid, dental pulp, bone, umbilical cord, adipose tissue
- Expansion in culture while maintainig multipotency
- (Trans-)differentiation into different cell types: osteoblasts, chondrocytes, adipocytes, myocytes, cardiomyocytes, hepatocytes, epithelial cells, endothelial cells, neurons
- Heterogeneity
  - International Society for Cellular Therapy:
  - Plastic-adherent in standard culture conditions
  - Expression of CD105, CD73, CD90
  - Lack of CD45, CD34, CD14, CD11b, CD79a, CD19, HLA-DR
  - Must be able to differentiate into osteoblasts, adipocytes and chondrobalsts in vitro

DeMiguel MP et al. Immunosuppressive properties of mesenchymal stem cells: advances and applications. Curr Mol Med 2012; 12:574-591.

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Background

- BMSCs injected into diabetic animals reversed diabetic phenotypes and improved glucose control
- Poor direct ß-cell differentiation -> other possible roles of BMSCs in pancreatic islet regeneration
- Introduction of transcription factor genes into cultured human BMSCs
  - Activation of genes related to the development and function of ß-cells
- PDX1:
  - Master gene in pancreas development
  - Crucial for early pancreas differentiation
- VEGF-A:
  - Important for intra-islet angiogenesis
  - Vascular membrane is a niche for insulin gene expression and ß-cell proliferation
- 3 treatment groups:
  - hBMSCs
  - hBMSCs expressing PDX1
  - hBMSCs expressing VEGF

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Methods 1

- Human BMSC Culture and expansion
  - hBMSCs from a single donor, passage #7
- Adenovirus production and cell transfection
  - cDNAs for human PDX1 and mouse VEGF165 were subcloned into AdenoX viral DNA vector
  - hBMSCs were transfected with adenovirus 2 days before transplantation
- Animal model and stem cell transplantation
  - NOD/SCID mice
  - 3 i.p. injections of streptozotocin
  - hBMSCs / hBMSCs-VEGF / hBMSCs-PDX1
  - Injection of 1x10^6 cells (on day 7) intracardially
- Blood glucose and serum insulin measurements
  - non-fasting mice daily for 1 week, then twice a week
  - Mouse insulin ÉLISA, human insulin ELISA

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Methods 2

- Immunohistochemical analyses
  - Mouse pancreatic tissues harvested 6 weeks after stem cell injection
- ß-cell count
- Phase contrast and confocal microscopy analyses
- rtPCR arrays
  - Pancreatic tissue

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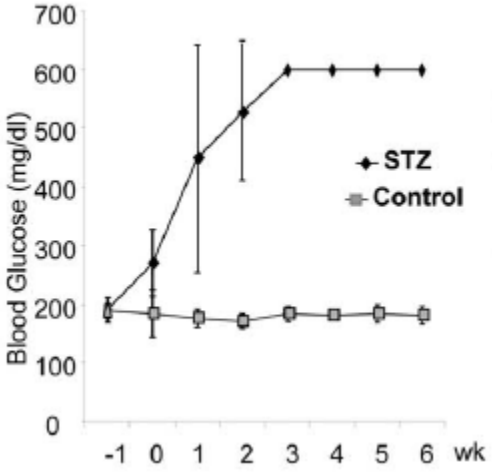
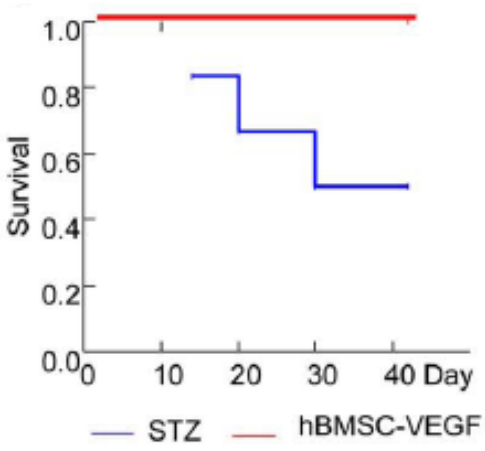
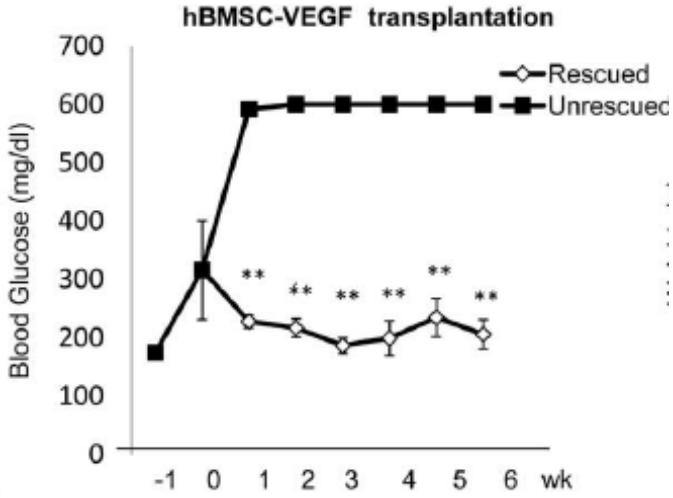
Results 1.1
hBMSCs-VEGF







- Mice treated with STZ developed hyperglycemia 6-7 days after STZ- injection
- High mortality rate of diabetic mice
- Reversion of hyperglycemia due to hBMSC-VEGF injection

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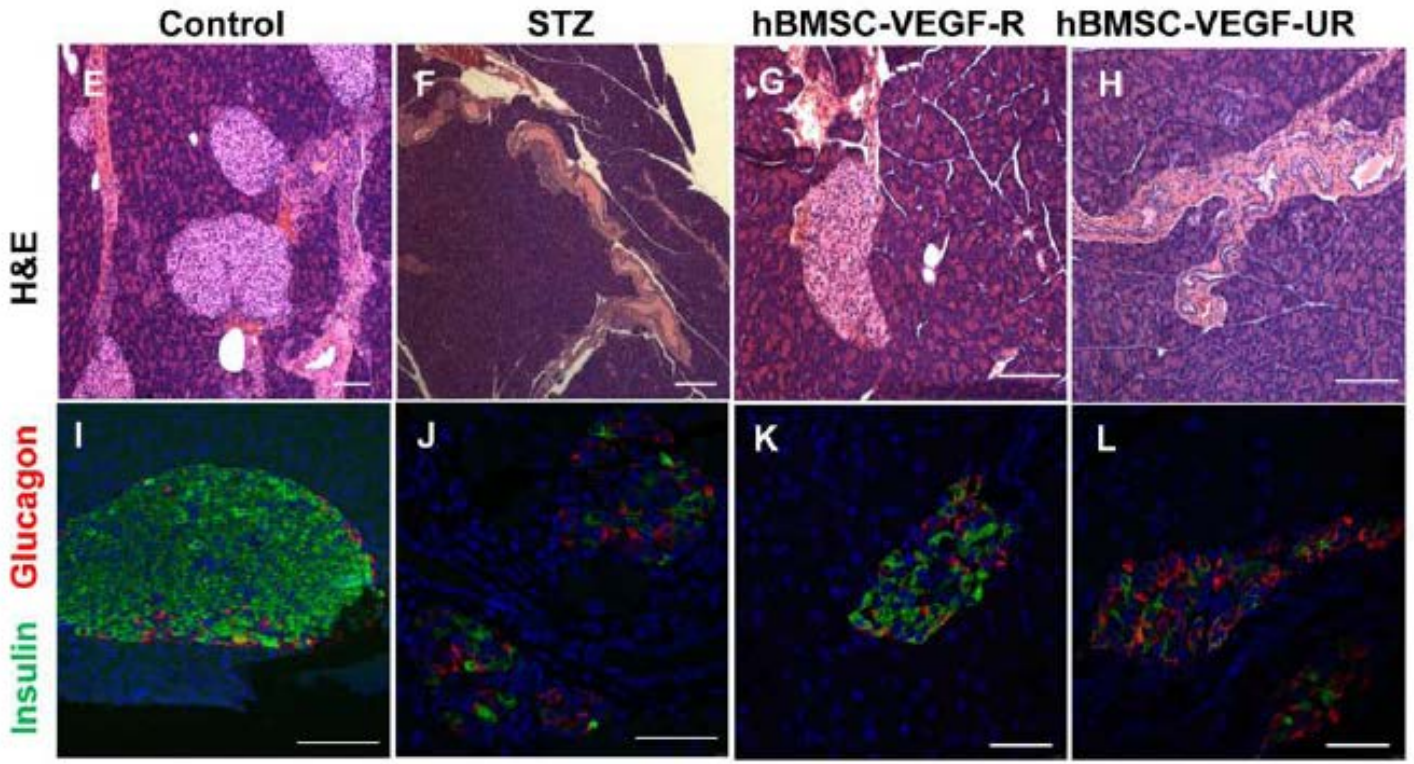
Results 1.2
hBMSCs-VEGF

Histological examination of the pancreatic islet morphology (6w after TX)



- Reduction of the number of insulin-expressing cells in STZ-induced diabetic mice
- Similar staining pattern in control mice and hBMSC-VEGF treated mice

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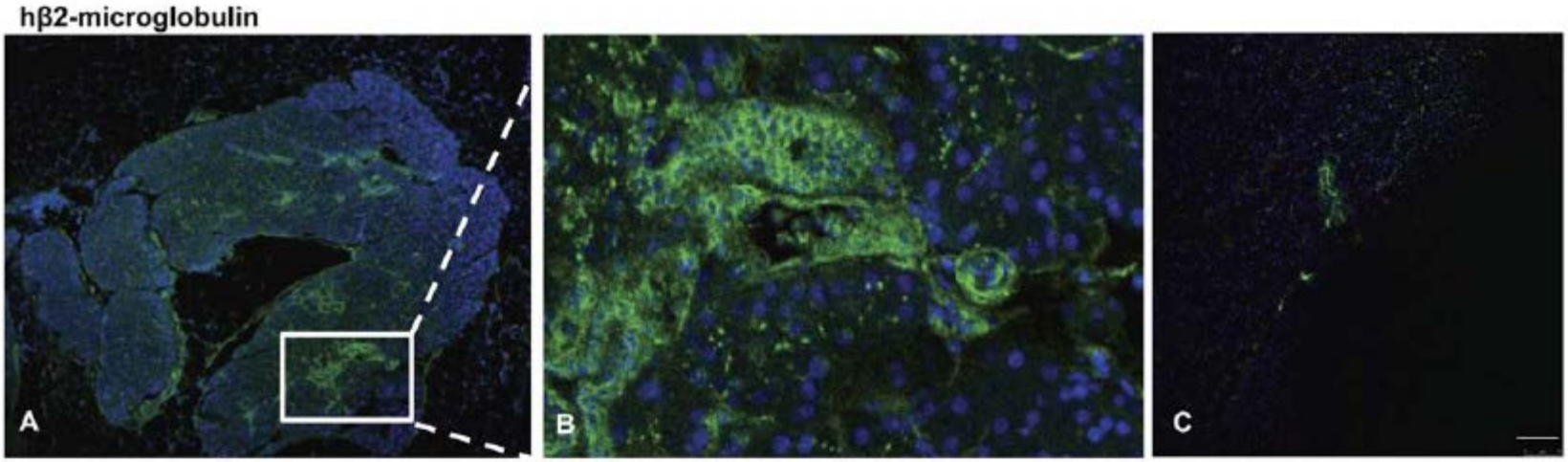
Results 1.3

hBMSCs-VEGF



Animal/Cells	Pancreas	Kidney	Liver
1/hBMSC-VEGF	0.2±0.05	ND	NA
2/hBMSC-VEGF	0.18±0.07	ND	NA
3/hBMSC-VEGF	0.025±0.005	0.004±0.001	ND
4/hBMSC-VEGF	0.03±0.007	0.015±0.007	ND
1/hBMSC	0.008±0.0005	ND	NA
2/hBMSC	0.0048±0.001	ND	NA
3/hBMSC	ND	ND	ND
4/hBMSC	ND	ND	NA
1–3/no cells	ND	ND	NA

Engraftment and survival of hBMSCs-VEGF in the mouse pancreas (6w after TX)

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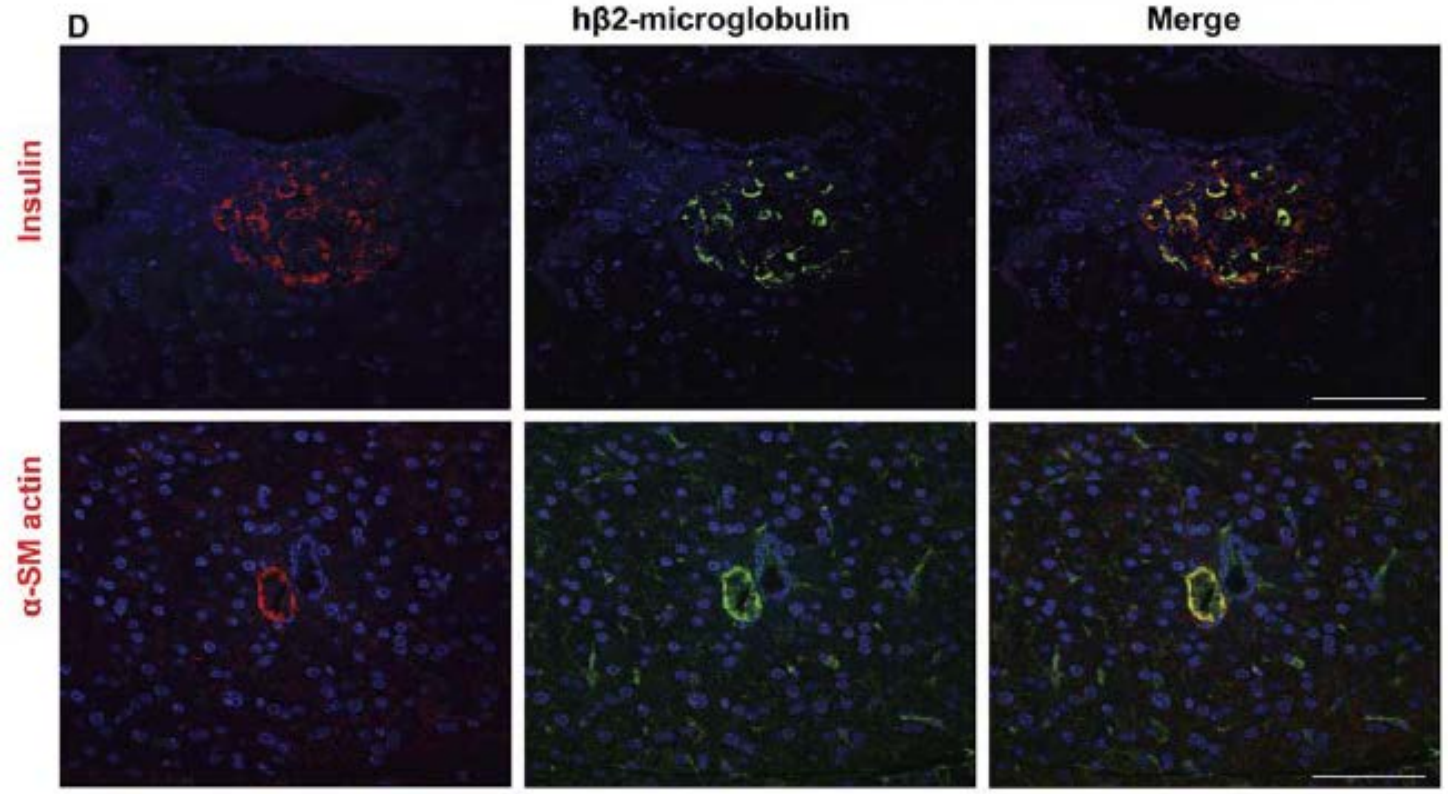
Results 1.4

hBMSCs-VEGF



hBMSCs-VEGF were able to differentiate into vessels and ß-cells

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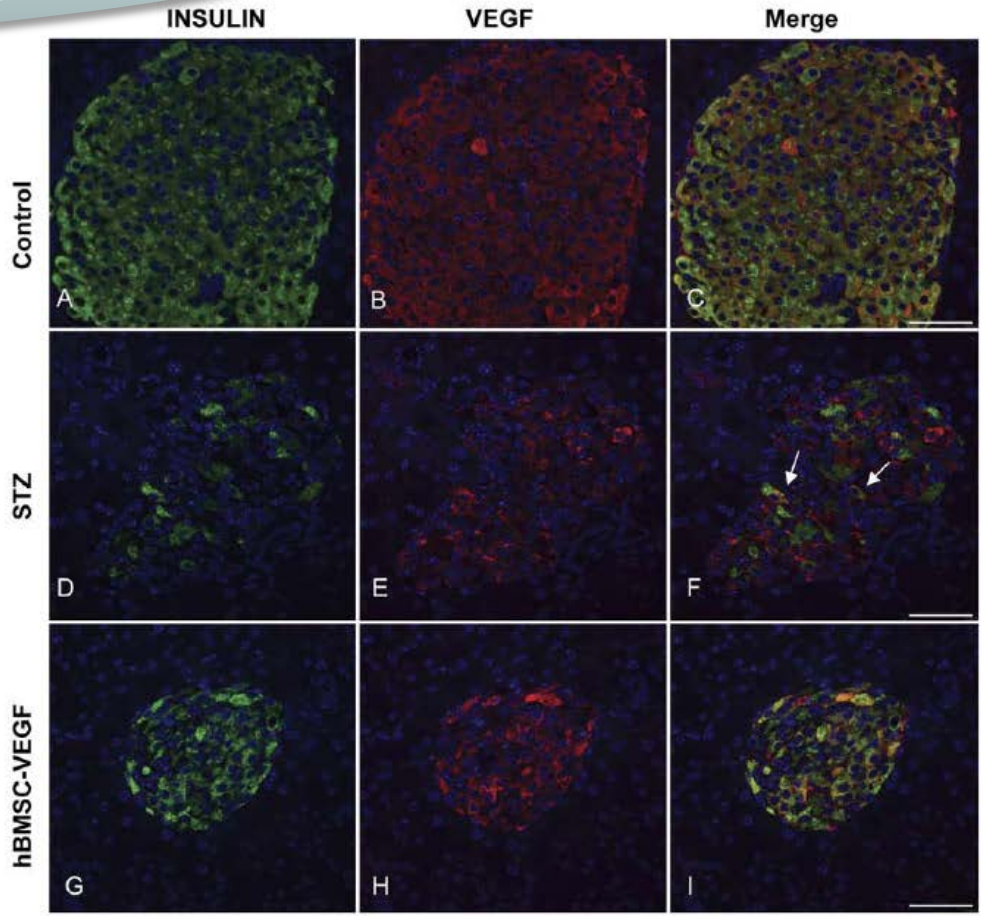
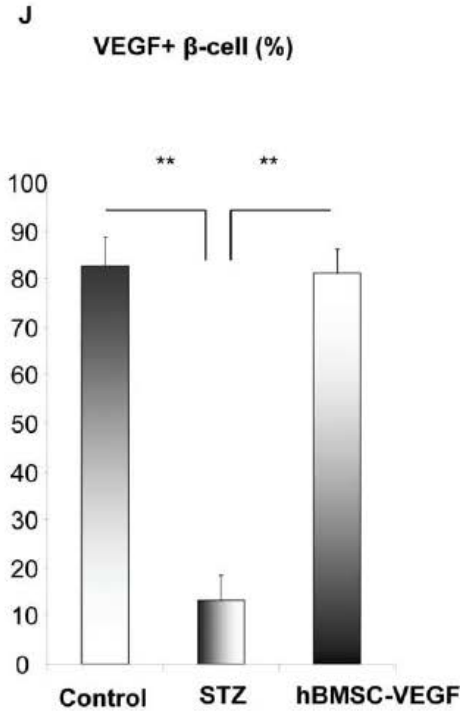
Results 1.5

hBMSCs-VEGF





- Reduction of VEGF expression in the ß-cells after induction of diabetes
- Restoration of VEGF expression after treatment with hBMSCs-VEGF

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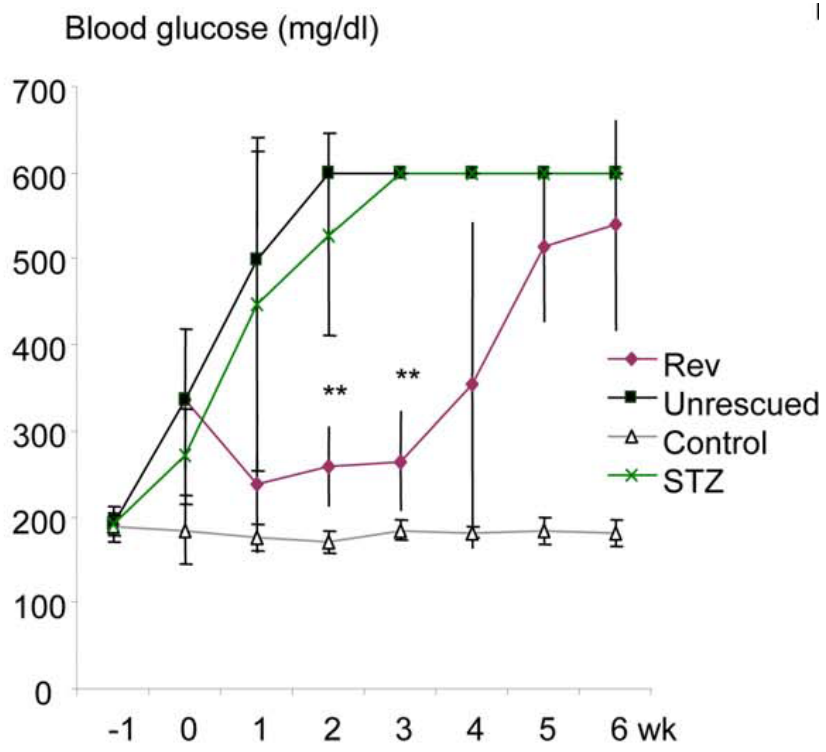
Results 2.1
hBMSCs-PDX1



- 50% of hBMSCs-PDX1 treated mice maintained severe hyperglycemia
- 50% showed reduction of hyperglycemia but again developed hyperglycemia after 2-3 weeks

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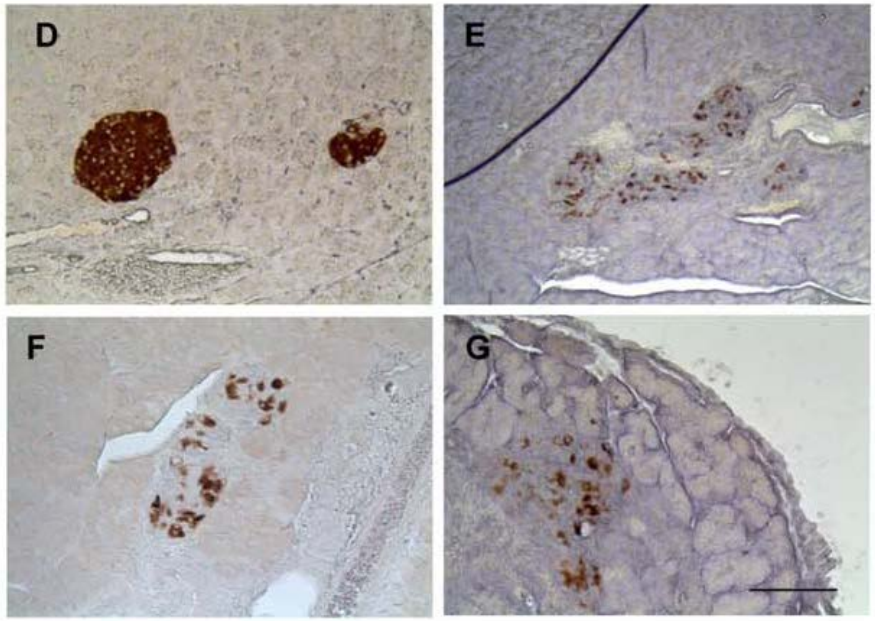
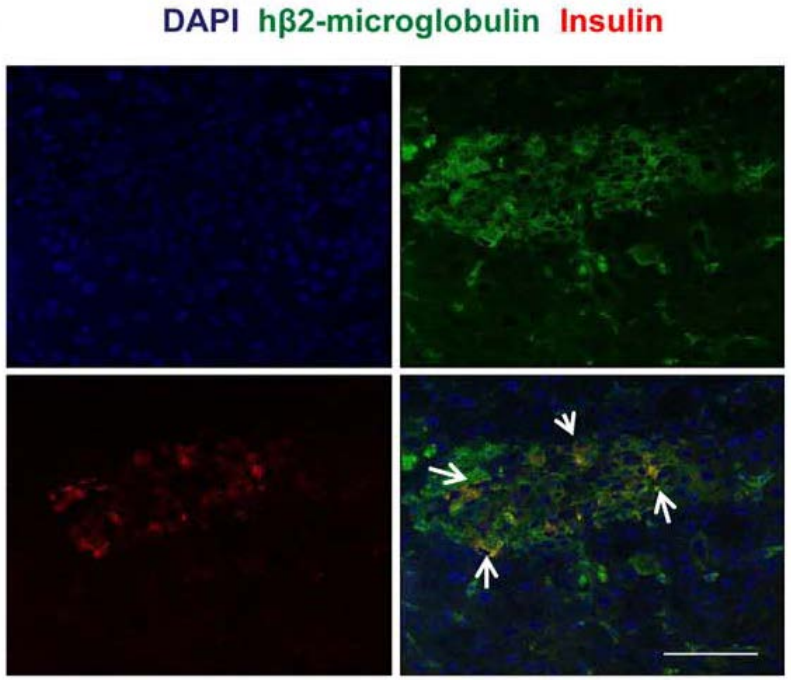
Results 2.2

hBMSCs-PDF1





Left:

- „Temporary reversed“ (F) and „unrescued“ (G) mice showed reduction of insulin expression in the pancreatic islets

Right:

- Engraftment of human cells in mouse pancreas

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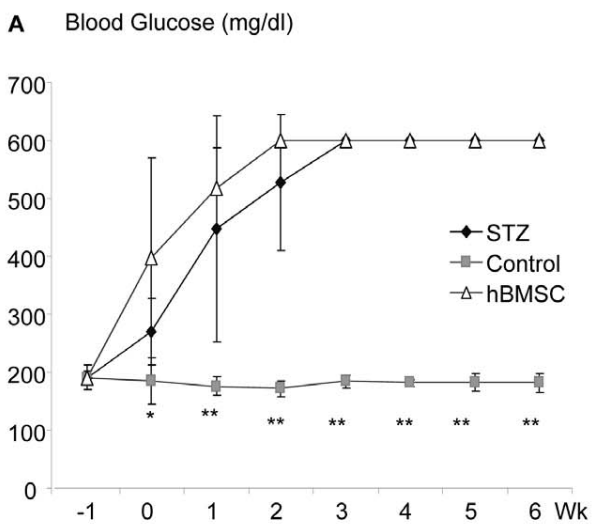
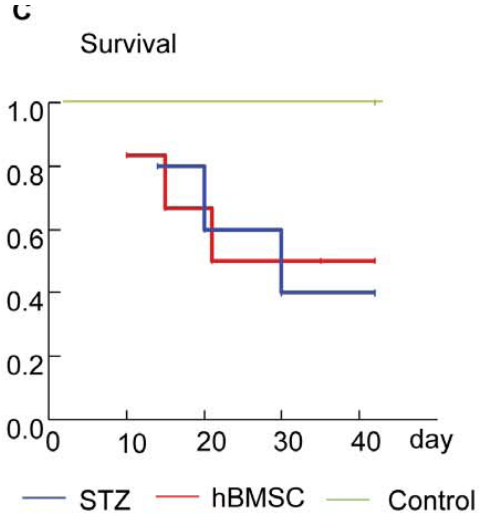
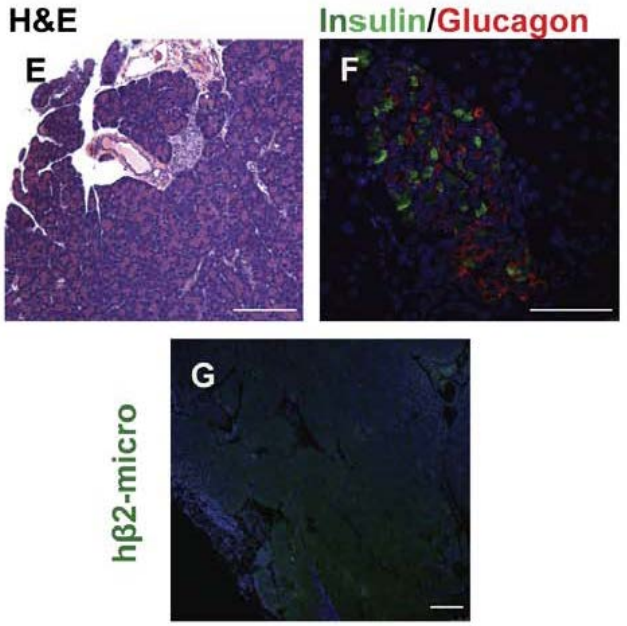
Results 3
hBMSCs







- hBMSCs without genetic modification did not ameliorate diabetic phenotypes
- survival rate similar to STZ-induced diabetic mice
- alteration of pancreatic islet morphology, inversion in the insulin/glucagon ratio, poor engraftment of hBMSCs in the pancreas

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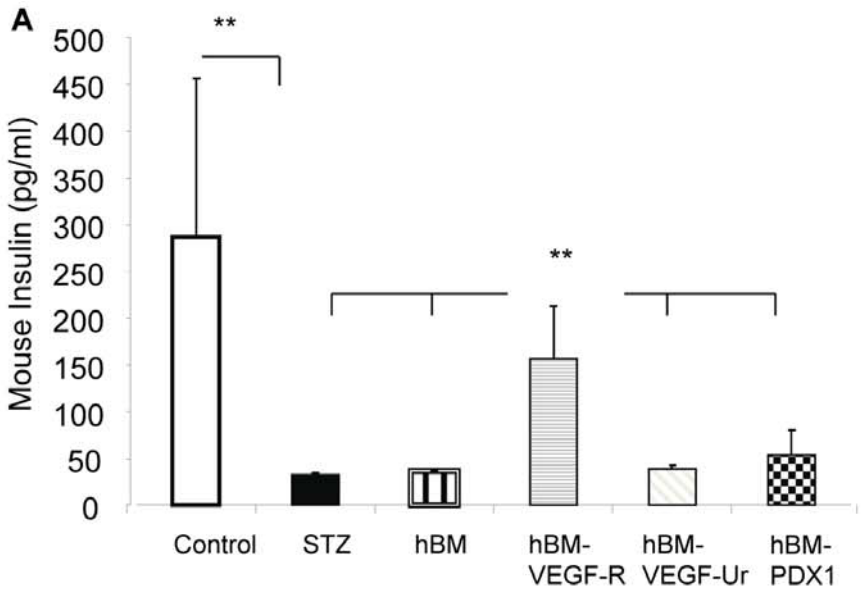
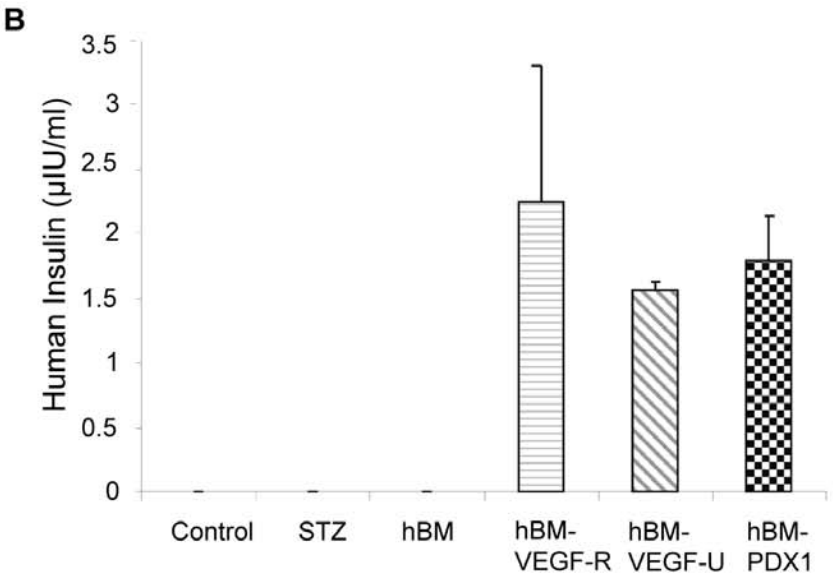
Results 4.1

Endogenous vs. Transplant-derived ß-cell differentiation





A: Only mice treated successfully with hBMSCs-VEGF showed significantly higher levels of mouse insulin compared with other groups

B: levels of human insulin were detectable in the therapy-groups

→ de novo differentiation of hBMSCs into ß-cells

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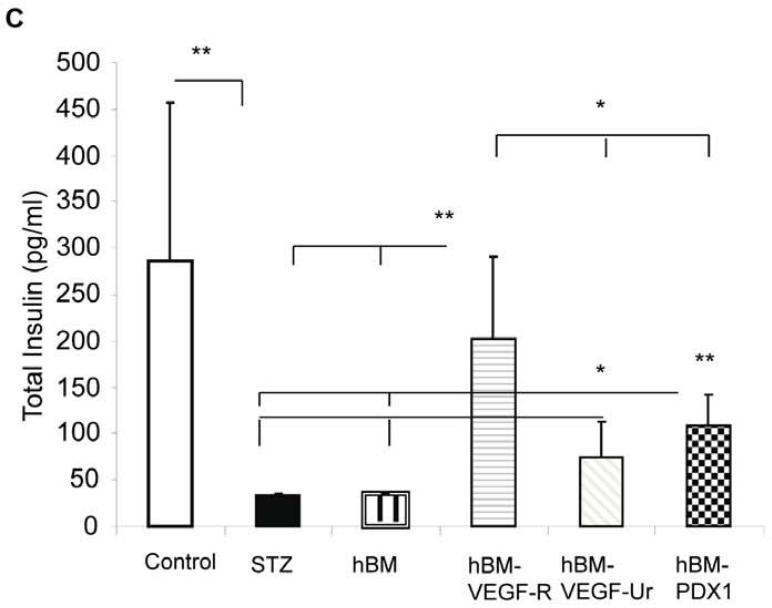
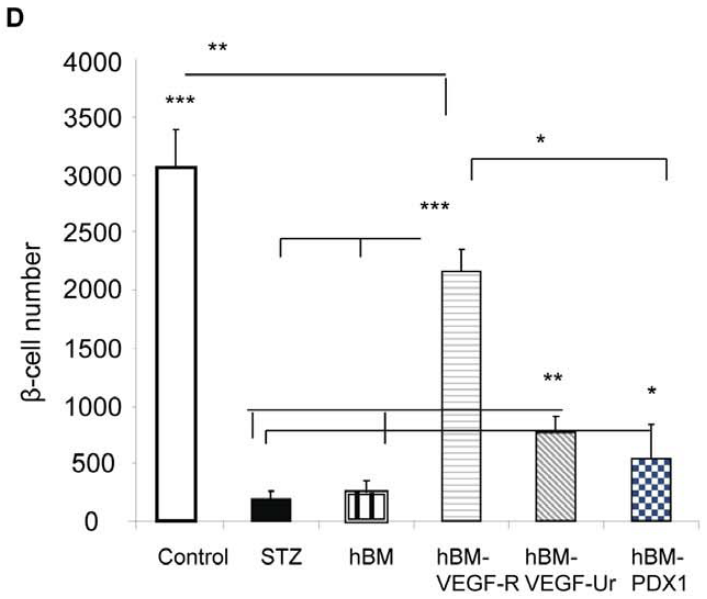
Results 4.2

Endogenous vs. Transplant-derived ß-cell differentiation





C: Levels of total serum insulin were higher in the therapy-groups

D: Number of ß-cells higher in therapy-groups (correlation with total insulin levels)

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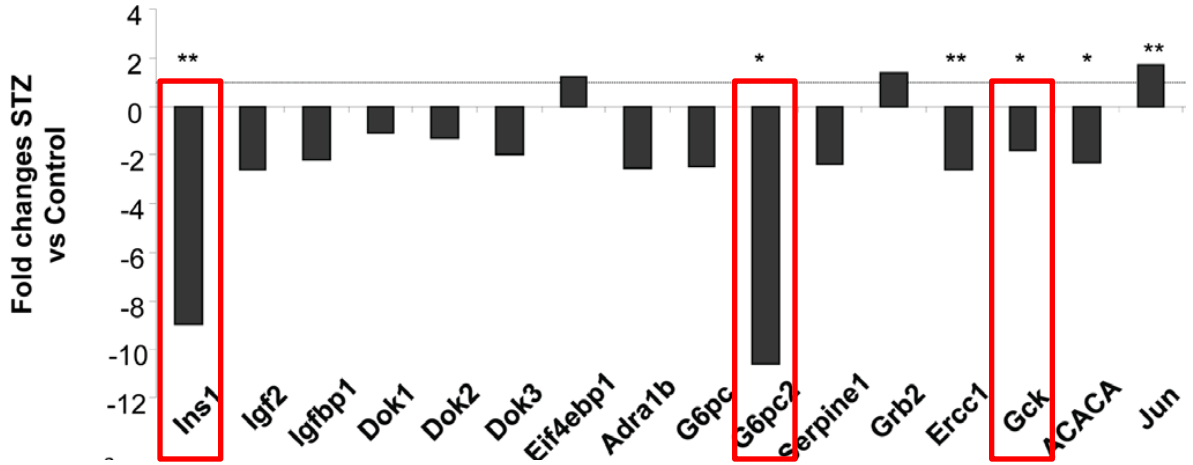
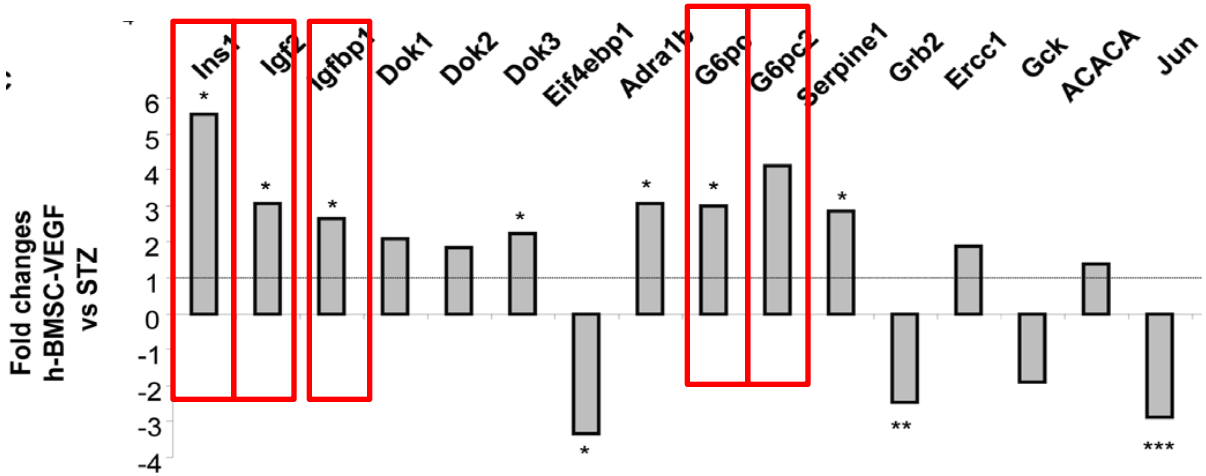
Results 5.1

Mechanisms of endogenous ß-cell recovery in hBMSCs-VEGF treated mice



Decreasing expression of genes related with insulin receptor signaling pathway in pancreases of diabetic mice



Up-regulation of genes involved in the insulin/IGF signaling pathway in pancreases of hBMSCs-VEGF treated mice

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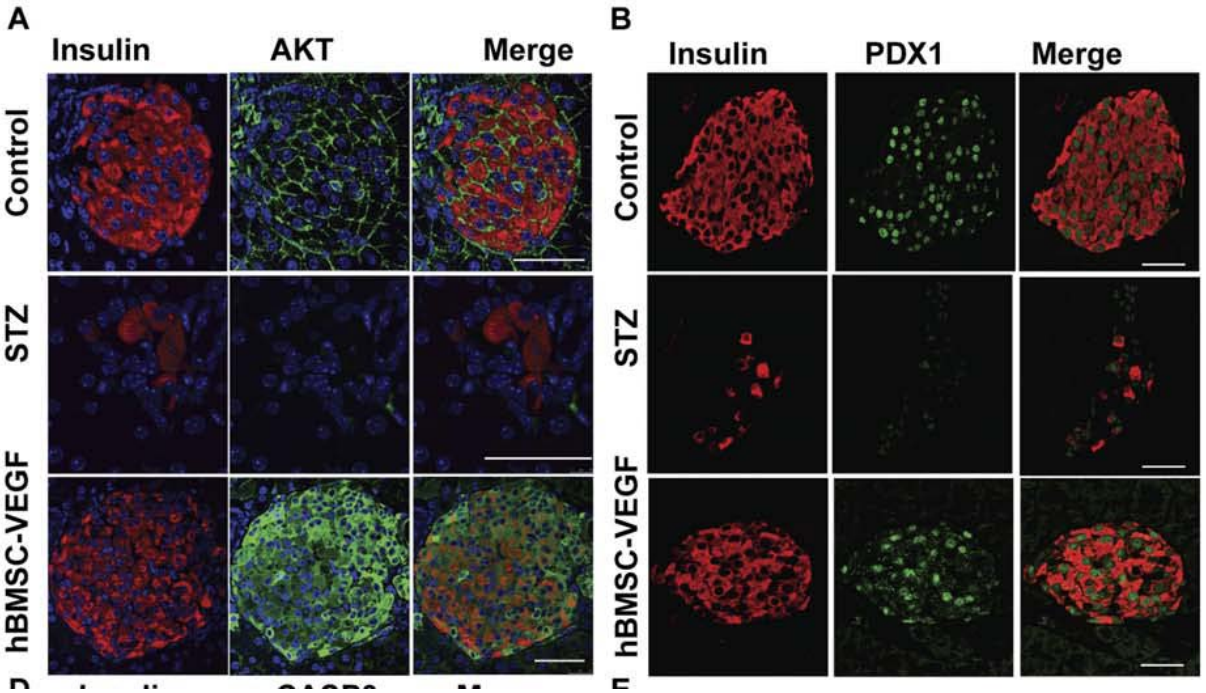
Results 5.2

Mechanisms of endogenous ß-cell recovery in hBMSCs-VEGF treated mice



AKT and downstream proteins required for ß-cell proliferation, differentiation and survival are highly expressed in hBMSCs-VEGF mice

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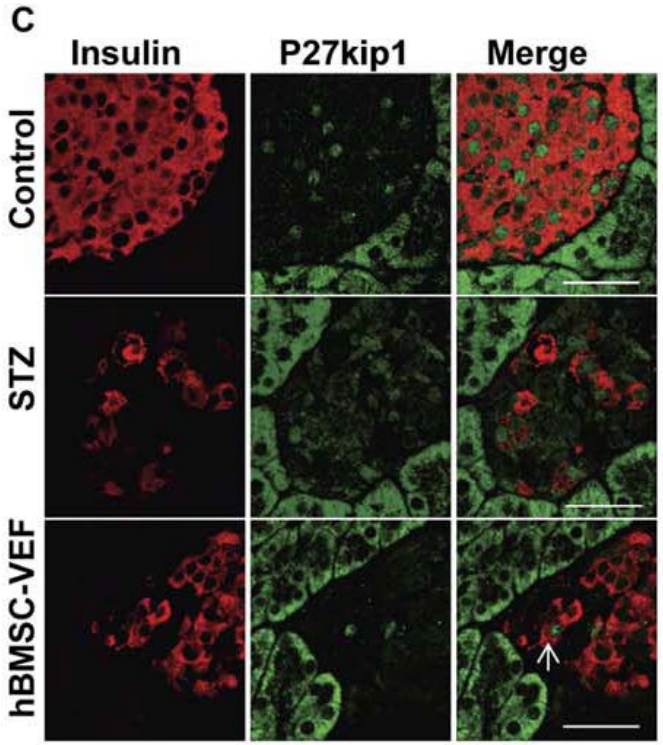
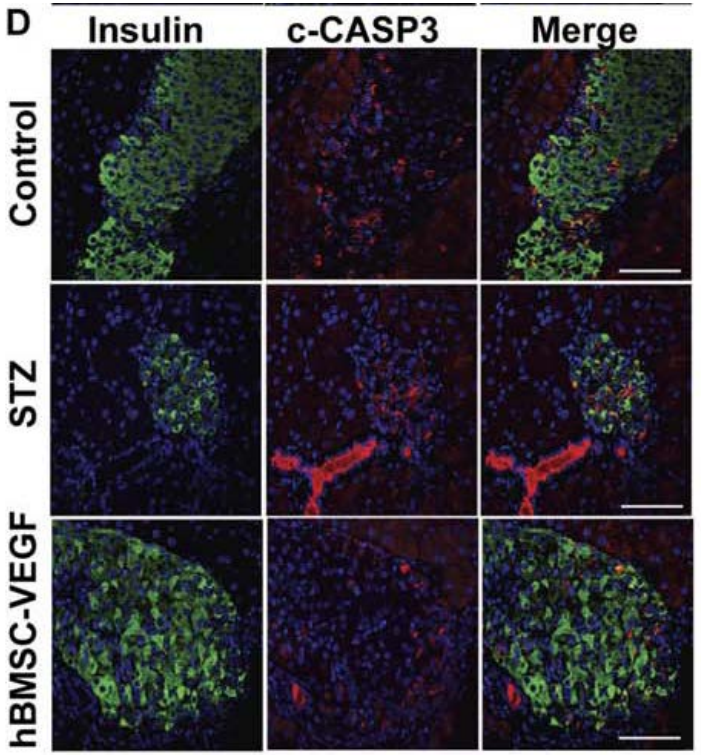
Results 5.3

Mechanisms of endogenous ß-cell recovery in hBMSCs-VEGF treated mice





- P27kip1 (cell cycle inhibitor protein negatively regulated through PI-3K/AKT) was upregulated in diabetic mice and downregulated in hBMSCs-VEGF mice
- c-CASP3 was highly increased in diabetic mice

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Discussion

- hBMSCs alone were not able to reverse hyperglycemia
- Recovery from diabetes following hBMSCs-VEGF injection
  - Engraftment of hBMSCs-VEGF in the pancreas of diabetic mice
  - Differentiation of hBMSCs-VEGF into blood vessels and ß-cells
  - Detectable levels of human insulin → chimerism
  - Higher levels of mouse insulin → endogenous ß-cell regeneration
- Only transient recovery from diabetes following hBMSCs-PDX1 injection
- Upregulatin of insulin receptor associated genes in hBMSCs-VEGF mice
- Upregulation of genes involved in the PI-3K/AKT pathway
  - Inhibition of apoptosis
  - ß-cell differentiation and proliferation through activation of PDX1 and inhibition of P27Kip1
  - Modulation of intra-islet angiogenesis → VEGF expression