

## SUPPLEMENTAL DIGITAL CONTENT

### EGF-GH AXIS IN RAT STEATOTIC AND NON-STEATOTIC LIVER

#### TRANSPLANTATION FROM BRAIN DEAD DONORS

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## SUPPLEMENTAL MATERIAL AND METHODS

Animals were randomly distributed into groups as described below.

### Experimental design

*Protocol 1. GH and EGF in steatotic and non-steatotic liver grafts from BD donors after LT*

Group 1. **Sham** (n=12). Six Ob and six Ln Zucker rats were anesthetized, ventilated, and maintained normotensive with saline infusion for 6 hours<sup>1,2</sup>.

Group 2. **LT** (n=24). Six Ob and six Ln Zucker rats were anesthetized, ventilated, and maintained normotensive with saline infusion for 6 hours. Then, steatotic and non-steatotic livers were flushed with University of Wisconsin (UW) solution, isolated, preserved in ice-cold UW solution for 6 hours, and implanted into 12 Ln Zucker rats<sup>1-3</sup>.

Group 3. **BD+LT** (n=24). Six Ob and six Ln Zucker rats were anesthetized and ventilated. After BD induction, rats were maintained normotensive with colloid infusion for 6 hours. Then, livers were flushed with UW solution, isolated, preserved in ice-cold UW solution for 6 hours, and implanted into 12 Ln Zucker rats<sup>1,2</sup>.

Group 4. **BD+EGF<sub>D</sub>+LT** (n=24). Same as group 3 but treated with a single dose of EGF (100 mg/kg, i.p.) just after induction of BD before livers were flushed and preserved in UW solution for 6 hours<sup>4</sup>.

Group 5. **BD+GH<sub>D</sub>+LT** (n=24). Same as group 3 but treated with a single dose of GH (1.5 mg/kg, i.p.) just after induction of BD before livers were flushed and preserved in UW solution for 6 hours<sup>5</sup>.

Group 6. **BD+EGF<sub>D</sub>+GH<sub>D</sub>+LT** (n=24). Same as group 3 but treated with a single dose of EGF (100 mg/kg, i.p.) and a single dose of GH (1.5 mg/kg, i.p.) just after induction of BD before livers were flushed and preserved in UW solution for 6 hours<sup>4,5</sup>.

*Protocol 2. GH and EGF in donors before retrieval of steatotic and non-steatotic livers from BD donors*

Group 7. **BD** (n=12): Six Ob and six Ln Zucker rats were anesthetized and ventilated. After BD induction, rats were maintained normotensive with colloid infusion for 6 hours<sup>1,2</sup>.

Group 8 **BD+EGF<sub>D</sub>** (n=12). Same as group 7 but treated with a single dose of EGF (100 mg/kg, i.p.) just after induction of BD<sup>4</sup>.

Group 9. **BD+GH<sub>D</sub>** (n=12). Same as group 7 but treated with a single dose of GH (1.5 mg/kg, i.p.) just after induction of BD<sup>5</sup>.

Group 10. **BD+EGF<sub>D</sub>+GH<sub>D</sub>** (n=12). Same as group 7 but treated with a single dose of EGF (100 mg/kg, i.p.) and a single dose of GH (1.5 mg/kg, i.p.) just after induction of BD<sup>4,5</sup>.

*Protocol 3. Effects of exogenous EGF-GH when these drugs are administered only in the recipient as well as in both the donor and recipient*

Group 11. BD+EGF<sub>R</sub>+LT (n=24). Same as group 3 but recipients were treated with a single dose of EGF (100 mg/kg, i.p.) at 10 min after reperfusion<sup>4</sup>.

Group 12. BD+EGF<sub>DR</sub>+LT (n=24). Same as group 3 but donors were treated with a single dose of EGF (100 mg/kg, i.p.) just after induction of BD before livers were flushed and preserved in UW solution for 6 hours and recipients were treated with a single dose of EGF (100 mg/kg, i.p.) at 10 min after reperfusion<sup>4</sup>.

Group 13. BD+GH<sub>R</sub>+LT (n=24). Same as group 3 but recipients were treated with a single dose of GH (1.5 mg/kg, i.p.) at 10 min after reperfusion<sup>5</sup>.

Group 14. BD+GH<sub>DR</sub>+LT (n=24). Same as group 3 but donors were treated with a single dose of GH (1.5 mg/kg, i.p.) just after induction of BD before livers were flushed and preserved in UW solution for 6 hours and recipients were treated with a single dose of GH (1.5 mg/kg, i.p.) at 10 min after reperfusion<sup>5</sup>.

Samples were collected from Sham rats and recipients at 4 hours after reperfusion in experimental groups 1-6, Protocol 1 and experimental groups 11-14, Protocol 3. For survival studies, animals were subjected to an intervention similar to that used for groups 2-6 of Protocol 1 and the survival of receptors was monitored for 14 days after liver surgery<sup>1,2</sup>. In addition, for groups 7-10 of Protocol 2, samples were taken from donors at 6 hours after normotensive BD induction. GH, somatostatin and ghrelin fluctuate according to feedings and circadian cycles<sup>6,7</sup>. To limit variations due to the timing of the measurement, organ harvesting and transplantation were performed in the morning or afternoon, which is the quiescent period in a rat's circadian rhythm. Blood and liver samples were taken for corresponding measurements during this time. Nevertheless, in accordance with preliminary studies from our group, the circadian rhythm did not affect the levels of the mentioned hormones in Ln and Ob rats. The conditions of the present study and the doses and pre-treatment times used for the different drugs were selected based on previous studies<sup>4,5</sup> as well as preliminary studies from by group.

### **Biochemical determinations**

Plasma transaminases, aspartate transaminase (AST) and alanine transaminase (ALT) were measured photometrically using standard procedures.

Plasma and liver GH, EGF, somatostatin, ghrelin and growth hormone releasing hormone (GHRH) were determined by enzyme-linked immune sorbent assay (Bionova Científica, Madrid, Spain) according to the manufacturer's instructions. Plasma and liver GH, EGF, somatostatin, ghrelin and growth hormone releasing hormone (GHRH) were determined by enzyme-linked immune sorbent assay (Bionova Científica, Madrid, Spain) according to the manufacturer's instructions. Plasma and liver homogenates were

added in the 96-well plates (100  $\mu$ L/well) and incubated at 37°C for 2 hours. After removing the liquid of each well, the plate was incubated with 100  $\mu$ L Biotin-antibody for 1 hour at 37°C. After washing, HRP-avidin was added and incubated for 1 hour at 37°C. Afterwards of removing the liquid and washing, 90  $\mu$ L of TMB was added to each well and incubated at 37°C for 15 -30 minutes, followed by the termination of the reaction using 50  $\mu$ L Stop Solution. A wavelength of 450 nm was used for the determination of absorbance.

Lipid peroxidation was determined by measuring the formation of malondialdehyde (MDA) as an indirect index of the oxidative injury induced by the reactive oxygen species<sup>8,9</sup>. Briefly, 0.5 ml of 0.5% butylated hydroxytoluene was added to 2 ml of liver homogenate to prevent lipid autoxidation. For protein precipitation, 2 ml of 20% trichloroacetic acid was added to 2 ml of homogenate. After mixing and centrifuging, 1 ml of 0.67% thiobarbiturate solution was added to the supernatant and boiled for 60 minutes. After cooling, optical density at 530 nm was assayed<sup>8</sup>.

Myeloperoxidase (MPO), as an index of neutrophil accumulation, was measured photometrically using 3,3',5,5'-tetramethyl-benzidine as a substrate. Liver samples were macerated with 0.5% hexadecyltrimethylammonium bromide in 50 mM phosphate buffer pH 6.0. Homogenates were then disrupted for 30 sec using a sonicator at 20% power and subsequently snap frozen in dry ice and thawed on three consecutive occasions before a final 30-sec sonication. Samples were incubated at 60°C for 2 hours and then spun down at 4000g for 12 minutes. Supernatants were collected for MPO assay. Enzyme activity was assessed photometrically at 630 nm. The assay mixture consisted in 20  $\mu$ l supernatant, 10  $\mu$ l tetramethylbenzidine (final concentration 1.6 mM) dissolved in DMSO, and 70  $\mu$ l H<sub>2</sub>O<sub>2</sub> (final concentration 3.0 mM) diluted in 80 mM

phosphate buffer pH 5.4. An enzyme unit is defined as the amount of enzyme that produces an increase of 1 absorbance unit per minute<sup>10</sup>.

Hepatic edema was measured as described elsewhere<sup>11</sup>. Briefly, tissue samples were weighed and then placed in an oven at 55°C until a constant weight was obtained. Edema was calculated by an increase in the wet-to-dry weight ratios.

### **Western blotting**

Liver tissue was homogenized in 10 mM Hepes, pH 7.6, 3 mM MgCl<sub>2</sub>, 40 mM KCl, 5% glycerol, 0.2% Nonidet P-40 and protease inhibitors and the lysate was centrifuged at 16,000g for 5 minutes. Liver homogenates containing equal amount of protein were mixed in Laemmli loading buffer, boiled for 5 minutes, separated on a sodium dodecyl sulfate 8-12% poly-acrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes<sup>8</sup>. After assessing transfer, the membranes were saturated in 4 mM Tris-HCl, pH 7.6, 30 mM NaCl (TBS) containing 20% non-fat milk and 0.1% Tween-80 and incubated over night at 4°C using antibodies against the following proteins: phosphoinositide-3-kinase (PI3K) (Cell Signaling Technology, Danvers, MA, USA); total and phosphorylated Akt (T-Akt and p-Akt, respectively) (Santa Cruz Biotechnology, Dallas, TX, USA); high mobility group protein B1 (HMGB1), cyclin D1 and  $\beta$ -Actin (Sigma-Aldrich, St Louis, MO, USA) to control equal protein loading; suppressors of cytokine signaling (SOCS) 1, 2 and 3 (Aviva Systems Biology, San Diego, CA, USA). Signals were detected by enhanced chemiluminescence and quantified with scanning densitometry relied on standard software (Quantity One; Bio-Rad Laboratories, Hercules, CA, USA)<sup>8</sup>.

### **Histology and Oil Red staining**

To assess the severity of hepatic injury, paraffin-embedded liver sections were stained with hematoxylin and eosin and blind histological scoring was performed by a board certified pathologist, using a point-counting method on an ordinal scale as follows: grade 0, minimal or no evidence of injury; grade 1, mild injury consisting of cytoplasmic vacuolation and focal nuclear pyknosis; grade 2, moderate to severe injury with extensive nuclear pyknosis, cytoplasmic hypereosinophilia, and loss of intercellular borders; grade 3, severe necrosis with disintegration of hepatic cords, hemorrhage, and neutrophil infiltration; and grade 4, very severe necrosis with disintegration of hepatic cords, hemorrhaging, and neutrophil infiltration<sup>2</sup>. Liver steatosis was visualized by Oil Red staining of liver cryosections. Liver tissues were frozen in Optimal Cutting Temperature (OCT) compounds. The sections were fixed with 10% formalin and the slides were placed in 100% propylene glycol, and stained in 0.5% Oil Red O solution in propylene glycol. The slides were transferred to an 85% propylene glycol solution and processed for hematoxylin counter staining<sup>12</sup>. At least 30 high-power fields were counted per slide.

### **Immunohistochemistry**

After fixation with 4% formalin/phosphate-buffered saline (PBS), paraffin-embedded, livers were sliced and immunostained using mouse monoclonal antibody anti proliferating cell nuclear antigen (PCNA) (DAKO, Santa Clara, CA, USA). Staining was developed with DAB, slides were counterstained with hematoxylin<sup>13</sup>. At least 30 high-power fields were counted per slide.

## **Statistics**

Data are expressed as means  $\pm$  standard error and were statistically analyzed via one-way analysis of variance, followed by post hoc Student-Newman-Keuls test. Survival was estimated using the Kaplan-Meier method and was statistically analyzed with a log-rank test.  $P < 0.05$  was considered significant.



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## SUPPLEMENTARY FIGURE LEGENDS

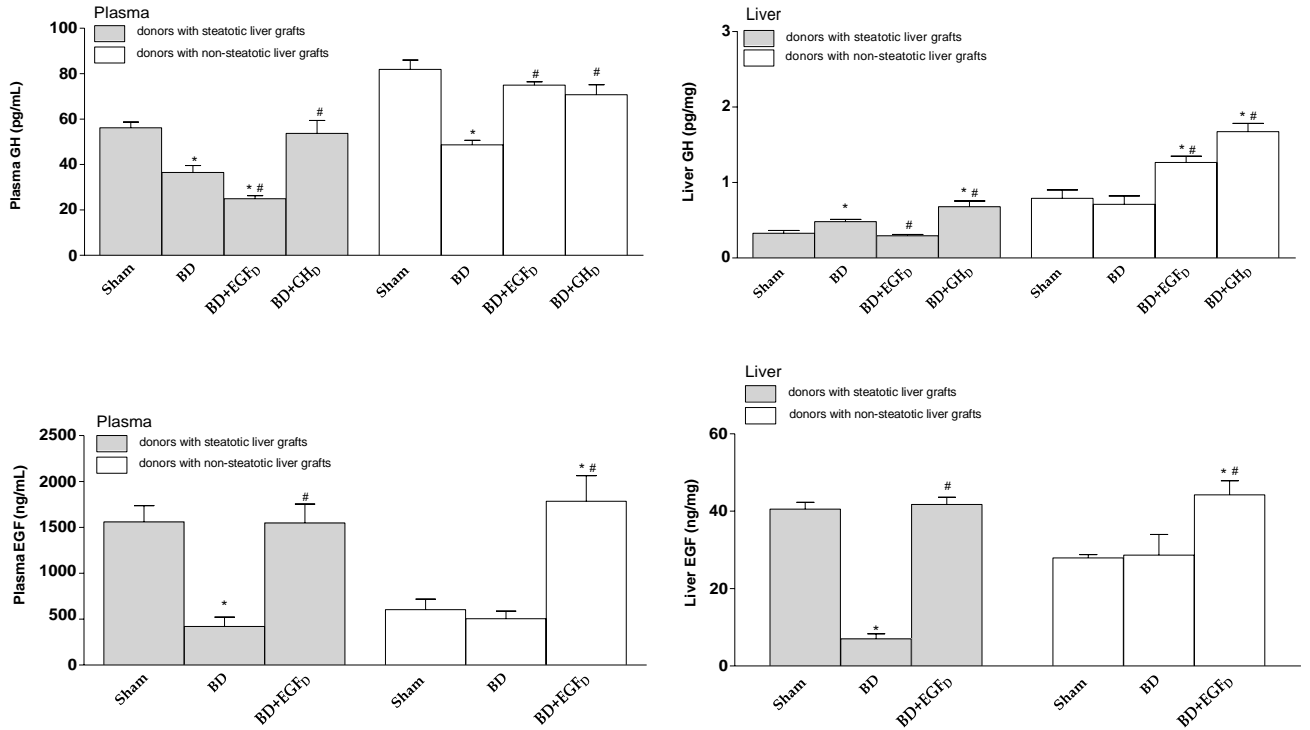
**Figure S1. Effects of growth hormone and epidermal growth factor on hepatic damage in donors before retrieval of liver grafts from BD donors.** (A) Levels of growth hormone (GH) and epidermal growth factor (EGF) in plasma and liver and (B) hepatic damage (plasma aspartate aminotransferase, AST and alanine aminotransferase, ALT) were evaluated in donors at 6 hours after BD induction and immediately before retrieval of the liver grafts from the donors. BD+EGF<sub>D</sub>: BD+EGF administration in the BD donor; BD+GH<sub>D</sub>: BD+GH administration in the BD donor. For A and B, there were six Ln and six Ob rats per group for each measurement \*p <0.05 vs. Sham; # <0.05 vs. BD.

**Figure S2. Effects of growth hormone and epidermal growth factor when these drugs are administered only in the recipient as well as in both the donor and recipient.** (A) Levels of growth hormone (GH) and epidermal growth factor (EGF) in liver were evaluated 4 hours after LT. (B) Hepatic damage (plasma aspartate aminotransferase, AST and alanine aminotransferase, ALT) were evaluated 4 hours after LT. (C) Hepatic regeneration (percentage of positive hepatocytes of PCNA and protein levels of cyclin D1) was evaluated 4 hours after LT. BD+EGF<sub>D</sub>+LT: BD+EGF administration in BD donor+LT; BD+EGF<sub>R</sub>+LT: BD+EGF administration in recipient+LT; BD+EGF<sub>DR</sub>+LT: BD+EGF administration in both BD donor and recipient+LT; BD+GH<sub>D</sub>+LT: BD+GH administration in the BD donor+LT; BD+GH<sub>R</sub>+LT: BD+GH administration in the recipient+LT; BD+GH<sub>DR</sub>+LT: BD+GH administration in both BD donor and recipient+LT. For A, B, C and D, there were six transplants with non-steatotic grafts and six transplants with non-steatotic grafts per group in each measurement. °p <0.05 vs. BD+LT; ^ <0.05 vs. BD+EGF<sub>D</sub>+LT.

## **SUPPLEMENTARY TABLE LEGEND**

**Table S1.** Significant p values for the experiments of the manuscript. Data were statistically analyzed via one-way analysis of variance, followed by post hoc Student-Newman-Keuls test. Survival was estimated with the Kaplan-Meier method and was statistically analyzed with a long-rank test.  $P < 0.05$  was considered significant.

## A. Growth hormone and epidermal growth factor protein levels



## B. Hepatic damage in steatotic and non-steatotic livers

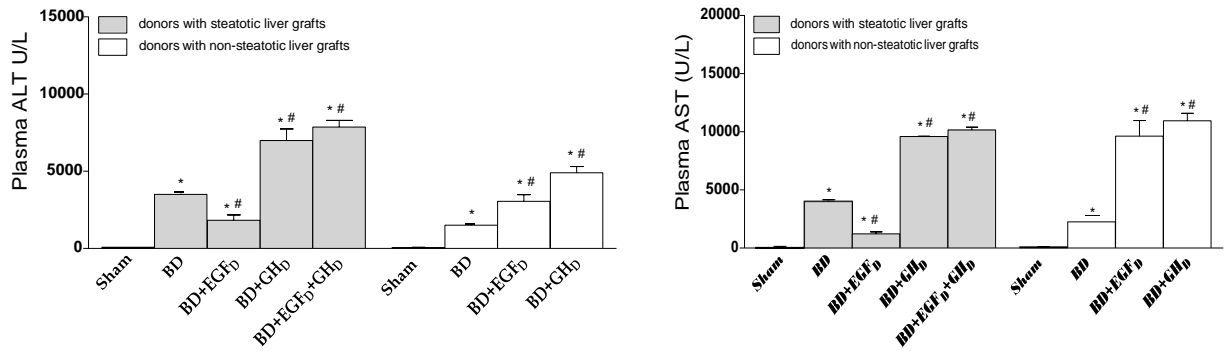
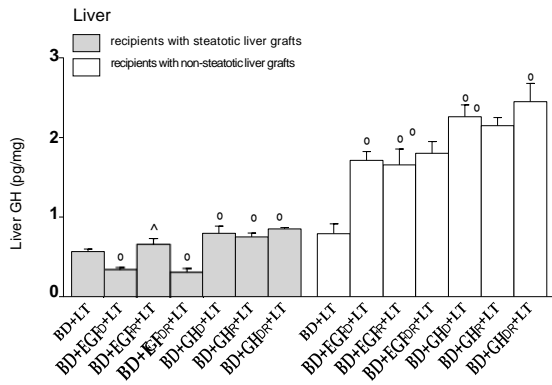
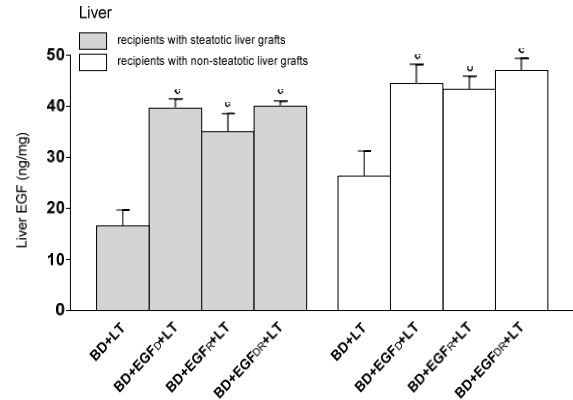


Figure S1

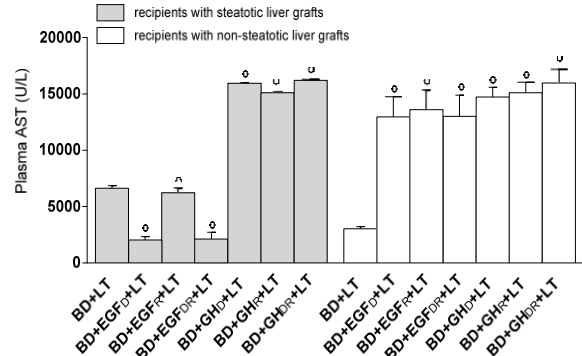
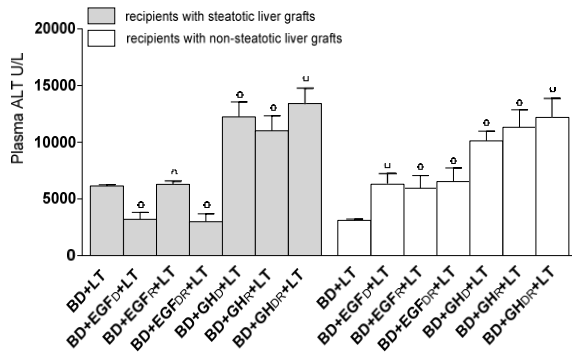
### A. Growth hormone protein levels



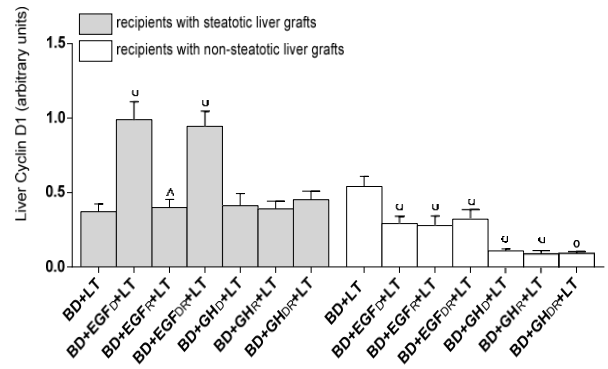
### B. Epidermal growth factor protein levels



### C. Damage in steatotic and non-steatotic livers



### D. Regeneration parameters in steatotic and non-steatotic livers



**Figure S2**

**Table S1. Significant p values for the experiments of the manuscript.**

Data were statistically analyzed via one-way analysis of variance, followed by post hoc Student-Newman-Keuls test. Survival was estimated with the Kaplan-Meier method and was statistically analyzed with a long-rank test.  $P < 0.05$  was considered significant.

<b>FIGURE 2</b>			
<b>Growth hormone protein levels in recipients with steatotic or non-steatotic liver grafts</b>			
<i>Plasma</i>			
<i>With steatotic livers grafts</i>		<i>With non-steatotic livers grafts</i>	
Experimental groups	P value	Experimental groups	P value
LT vs Sham	NS	LT vs Sham	NS
BD+LT vs Sham	<0.05	BD+LT vs Sham	<0.001
BD+LT vs LT	<0.05	BD+LT vs LT	<0.01
BD+EGF <sub>D</sub> +LT vs Sham	<0.001	BD+EGF <sub>D</sub> +LT vs Sham	NS
BD+EGF <sub>D</sub> +LT vs LT	<0.001	BD+EGF <sub>D</sub> +LT vs LT	NS
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.05	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001
BD+GH <sub>D</sub> +LT vs Sham	NS	BD+GH <sub>D</sub> +LT vs Sham	NS
BD+GH <sub>D</sub> +LT vs LT	NS	BD+GH <sub>D</sub> +LT vs LT	NS
BD+GH <sub>D</sub> +LT vs BD+LT	<0.05	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001
<i>Liver</i>			
<i>With steatotic livers grafts</i>		<i>With non-steatotic livers grafts</i>	
Experimental groups	P value	Experimental groups	P value
LT vs Sham	NS	LT vs Sham	NS
BD+LT vs Sham	<0.05	BD+LT vs Sham	NS
BD+LT vs LT	<0.01	BD+LT vs LT	NS
BD+EGF <sub>D</sub> +LT vs Sham	NS	BD+EGF <sub>D</sub> +LT vs Sham	<0.001
BD+EGF <sub>D</sub> +LT vs LT	NS	BD+EGF <sub>D</sub> +LT vs LT	<0.001
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.05	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001
BD+GH <sub>D</sub> +LT vs Sham	<0.001	BD+GH <sub>D</sub> +LT vs Sham	<0.001
BD+GH <sub>D</sub> +LT vs LT	<0.001	BD+GH <sub>D</sub> +LT vs LT	<0.001

BD+GH <sub>D</sub> +LT vs BD+LT	<0.01	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001
<b>Epidermal Growth Factor protein levels in recipients with steatotic or non-steatotic liver grafts</b>			
<i>Plasma</i>			
<i>With steatotic livers grafts</i>		<i>With non-steatotic livers grafts</i>	
Experimental groups	P value	Experimental groups	P value
LT vs Sham	NS	LT vs Sham	NS
BD+LT vs Sham	<0.01	BD+LT vs Sham	NS
BD+LT vs LT	<0.01	BD+LT vs LT	NS
BD+EGF <sub>D</sub> +LT vs Sham	NS	BD+EGF <sub>D</sub> +LT vs Sham	<0.001
BD+EGF <sub>D</sub> +LT vs LT	NS	BD+EGF <sub>D</sub> +LT vs LT	<0.01
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.01	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.01
<i>Liver</i>			
<i>With steatotic livers grafts</i>		<i>With non-steatotic livers grafts</i>	
Experimental groups	P value	Experimental groups	P value
LT vs Sham	NS	LT vs Sham	NS
BD+LT vs Sham	<0.001	BD+LT vs Sham	NS
BD+LT vs LT	<0.001	BD+LT vs LT	NS
BD+EGF <sub>D</sub> +LT vs Sham	NS	BD+EGF <sub>D</sub> +LT vs Sham	<0.01
BD+EGF <sub>D</sub> +LT vs LT	NS	BD+EGF <sub>D</sub> +LT vs LT	<0.01
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.01
<b>Growth hormone releasing hormone, somatostatin, and ghrelin protein levels in recipients with steatotic or non-steatotic liver grafts</b>			
<i>Plasma Growth hormone releasing hormone</i>			
<i>With steatotic livers grafts</i>		<i>With non-steatotic livers grafts</i>	
Experimental groups	P value	Experimental groups	P value
LT vs Sham	NS	LT vs Sham	NS
BD+LT vs Sham	NS	BD+LT vs Sham	NS
BD+LT vs LT	NS	BD+LT vs LT	NS
BD+EGF <sub>D</sub> +LT vs Sham	NS	BD+EGF <sub>D</sub> +LT vs Sham	NS



BD+EGF <sub>D</sub> +LT vs LT	NS	BD+EGF <sub>D</sub> +LT vs LT	NS
BD+EGF <sub>D</sub> +LT vs BD+LT	NS	BD+EGF <sub>D</sub> +LT vs BD+LT	NS
<b><i>Plasma Somatostatin</i></b>			
<b><i>With steatotic livers grafts</i></b>		<b><i>With non-steatotic livers grafts</i></b>	
Experimental groups	P value	Experimental groups	P value
LT vs Sham	NS	LT vs Sham	NS
BD+LT vs Sham	NS	BD+LT vs Sham	NS
BD+LT vs LT	NS	BD+LT vs LT	NS
BD+EGF <sub>D</sub> +LT vs Sham	<0.01	BD+EGF <sub>D</sub> +LT vs Sham	NS
BD+EGF <sub>D</sub> +LT vs LT	<0.01	BD+EGF <sub>D</sub> +LT vs LT	NS
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.01	BD+EGF <sub>D</sub> +LT vs BD+LT	NS
<b><i>Plasma Ghrelin</i></b>			
<b><i>With steatotic livers grafts</i></b>		<b><i>With non-steatotic livers grafts</i></b>	
Experimental groups	P value	Experimental groups	P value
LT vs Sham	NS	LT vs Sham	NS
BD+LT vs Sham	NS	BD+LT vs Sham	NS
BD+LT vs LT	NS	BD+LT vs LT	NS
BD+EGF <sub>D</sub> +LT vs Sham	NS	BD+EGF <sub>D</sub> +LT vs Sham	<0.001
BD+EGF <sub>D</sub> +LT vs LT	NS	BD+EGF <sub>D</sub> +LT vs LT	<0.001
BD+EGF <sub>D</sub> +LT vs BD+LT	NS	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001
<b>FIGURE 3</b>			
<b>Damage in recipients with steatotic or non-steatotic liver grafts</b>			
<b><i>Plasma ALT</i></b>			
<b><i>With steatotic livers grafts</i></b>		<b><i>With non-steatotic livers grafts</i></b>	
Experimental groups	P value	Experimental groups	P value
LT vs Sham	<0.001	LT vs Sham	<0.001
BD+LT vs Sham	<0.001	BD+LT vs Sham	<0.01
BD+LT vs LT	<0.05	BD+LT vs LT	<0.001
BD+EGF <sub>D</sub> +LT vs Sham	<0.01	BD+EGF <sub>D</sub> +LT vs Sham	<0.001
BD+EGF <sub>D</sub> +LT vs LT	NS	BD+EGF <sub>D</sub> +LT vs LT	<0.001

BD+EGF <sub>D</sub> +LT vs BD+LT	<0.05	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001
BD+GH <sub>D</sub> +LT vs Sham	<0.001	BD+GH <sub>D</sub> +LT vs Sham	<0.001
BD+GH <sub>D</sub> +LT vs LT	<0.001	BD+GH <sub>D</sub> +LT vs LT	<0.001
BD+GH <sub>D</sub> +LT vs BD+LT	<0.001	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>D</sub> +GH <sub>D</sub> +LT vs Sham	<0.001		
BD+EGF <sub>D</sub> +GH <sub>D</sub> +LT vs LT	<0.001		
BD+EGF <sub>D</sub> +GH <sub>D</sub> +LT vs BD+LT	<0.001		
<b><i>Plasma AST</i></b>			
<b><i>With steatotic livers grafts</i></b>		<b><i>With non-steatotic livers grafts</i></b>	
Experimental groups	P value	Experimental groups	P value
LT vs Sham	<0.001	LT vs Sham	<0.001
BD+LT vs Sham	<0.001	BD+LT vs Sham	<0.001
BD+LT vs LT	<0.001	BD+LT vs LT	<0.001
BD+EGF <sub>D</sub> +LT vs Sham	<0.001	BD+EGF <sub>D</sub> +LT vs Sham	<0.001
BD+EGF <sub>D</sub> +LT vs LT	<0.001	BD+EGF <sub>D</sub> +LT vs LT	<0.001
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001
BD+GH <sub>D</sub> +LT vs Sham	<0.001	BD+GH <sub>D</sub> +LT vs Sham	<0.001
BD+GH <sub>D</sub> +LT vs LT	<0.001	BD+GH <sub>D</sub> +LT vs LT	<0.001
BD+GH <sub>D</sub> +LT vs BD+LT	<0.001	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>D</sub> +GH <sub>D</sub> +LT vs Sham	<0.001		
BD+EGF <sub>D</sub> +GH <sub>D</sub> +LT vs LT	<0.001		
BD+EGF <sub>D</sub> +GH <sub>D</sub> +LT vs BD+LT	<0.001		
<b><i>Liver Damage Score</i></b>			
<b><i>With steatotic livers grafts</i></b>		<b><i>With non-steatotic livers grafts</i></b>	
Experimental groups	P value	Experimental groups	P value
BD+LT vs LT	<0.001	BD+LT vs LT	<0.01
BD+EGF <sub>D</sub> +LT vs LT	<0.01	BD+EGF <sub>D</sub> +LT vs LT	<0.001
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.05
BD+GH <sub>D</sub> +LT vs LT	<0.001	BD+GH <sub>D</sub> +LT vs LT	<0.001
BD+GH <sub>D</sub> +LT vs BD+LT	<0.05	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001

BD+EGF <sub>D</sub> +GH <sub>D</sub> +LT vs LT	<0.001		
BD+EGF <sub>D</sub> +GH <sub>D</sub> +LT vs BD+LT	<0.01		
<b>Regeneration parameters in recipients with steatotic or non-steatotic liver grafts</b>			
<i>% PCNA positive-hepatocytes</i>			
<i>With steatotic livers grafts</i>		<i>With non-steatotic livers grafts</i>	
Experimental groups	P value	Experimental groups	P value
BD+LT vs LT	<0.001	BD+LT vs LT	<0.001
BD+EGF <sub>D</sub> +LT vs LT	NS	BD+EGF <sub>D</sub> +LT vs LT	<0.001
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001
BD+GH <sub>D</sub> +LT vs LT	<0.001	BD+GH <sub>D</sub> +LT vs LT	<0.001
BD+GH <sub>D</sub> +LT vs BD+LT	NS	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>D</sub> +GH <sub>D</sub> +LT vs LT	<0.001		
BD+EGF <sub>D</sub> +GH <sub>D</sub> +LT vs BD+LT	<0.05		
<i>Liver Cyclin D1</i>			
<i>With steatotic livers grafts</i>		<i>With non-steatotic livers grafts</i>	
Experimental groups	P value	Experimental groups	P value
BD+LT vs LT	<0.001	BD+LT vs LT	<0.001
BD+EGF <sub>D</sub> +LT vs LT	NS	BD+EGF <sub>D</sub> +LT vs LT	<0.001
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.01
BD+GH <sub>D</sub> +LT vs LT	<0.001	BD+GH <sub>D</sub> +LT vs LT	<0.001
BD+GH <sub>D</sub> +LT vs BD+LT	NS	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>D</sub> +GH <sub>D</sub> +LT vs LT	<0.001		
BD+EGF <sub>D</sub> +GH <sub>D</sub> +LT vs BD+LT	NS		
<b>Survival of recipients with steatotic or non-steatotic liver grafts</b>			
<i>With steatotic livers grafts</i>		<i>With non-steatotic livers grafts</i>	
Experimental groups	P value	Experimental groups	P value
BD+LT vs LT	<0.05	BD+LT vs LT	<0.05
BD+EGF <sub>D</sub> +LT vs LT	NS	BD+EGF <sub>D</sub> +LT vs LT	<0.01
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.05	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.05
BD+GH <sub>D</sub> +LT vs LT	NS	BD+GH <sub>D</sub> +LT vs LT	<0.001

BD+GH <sub>D</sub> +LT vs BD+LT	<0.05	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>D</sub> +GH <sub>D</sub> +LT vs LT	NS		
BD+EGF <sub>D</sub> +GH <sub>D</sub> +LT vs BD+LT	<0.05		
<b>FIGURE 6</b>			
<b>PI3K/Akt pathway in recipients with steatotic liver grafts</b>			
<i>Liver PI3K</i>		<i>Liver p-Akt</i>	
Experimental groups	P value	Experimental groups	P value
BD+LT vs LT	<0.001	BD+LT vs LT	<0.001
BD+EGF <sub>D</sub> +LT vs LT	NS	BD+EGF <sub>D</sub> +LT vs LT	NS
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001
BD+GH <sub>D</sub> +LT vs LT	<0.001	BD+GH <sub>D</sub> +LT vs LT	<0.001
BD+GH <sub>D</sub> +LT vs BD+LT	NS	BD+GH <sub>D</sub> +LT vs BD+LT	NS
<b>SOCS1, SOCS2 and SOCS3 levels in recipients with steatotic liver grafts</b>			
<i>Liver SOCS1</i>		<i>Liver SOCS2</i>	
Experimental groups	P value	Experimental groups	P value
BD+LT vs LT	<0.001	BD+LT vs LT	NS
BD+EGF <sub>D</sub> +LT vs LT	NS	BD+EGF <sub>D</sub> +LT vs LT	NS
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>D</sub> +LT vs BD+LT	NS
BD+GH <sub>D</sub> +LT vs LT	<0.001	BD+GH <sub>D</sub> +LT vs LT	NS
BD+GH <sub>D</sub> +LT vs BD+LT	NS	BD+GH <sub>D</sub> +LT vs BD+LT	NS
<i>Liver SOCS3</i>			
Experimental groups	P value		
BD+LT vs LT	<0.001		
BD+EGF <sub>D</sub> +LT vs LT	NS		
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001		
BD+GH <sub>D</sub> +LT vs LT	<0.001		
BD+GH <sub>D</sub> +LT vs BD+LT	NS		
<b>Inflammatory response in recipients with steatotic liver grafts</b>			
<i>Liver HMGB1</i>		<i>Liver MDA</i>	
Experimental groups	P value	Experimental groups	P value

BD+LT vs LT	<0.001	BD+LT vs LT	<0.01
BD+EGF <sub>D</sub> +LT vs LT	NS	BD+EGF <sub>D</sub> +LT vs LT	NS
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.05	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.01
BD+GH <sub>D</sub> +LT vs LT	<0.001	BD+GH <sub>D</sub> +LT vs LT	<0.001
BD+GH <sub>D</sub> +LT vs BD+LT	NS	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001
<b><i>Liver MPO</i></b>		<b><i>Wet to dry weight ratio</i></b>	
Experimental groups	P value	Experimental groups	P value
BD+LT vs LT	<0.001	BD+LT vs LT	<0.001
BD+EGF <sub>D</sub> +LT vs LT	NS	BD+EGF <sub>D</sub> +LT vs LT	NS
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001
BD+GH <sub>D</sub> +LT vs LT	<0.001	BD+GH <sub>D</sub> +LT vs LT	<0.001
BD+GH <sub>D</sub> +LT vs BD+LT	<0.01	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001
<b>FIGURE S1</b>			
<b>Growth hormone and epidermal growth factor protein levels in donors with steatotic liver grafts</b>			
<b><i>Plasma Growth hormone</i></b>			
<b><i>With steatotic livers grafts</i></b>		<b><i>With non-steatotic livers grafts</i></b>	
Experimental groups	P value	Experimental groups	P value
BD vs Sham	<0.01	BD vs Sham	<0.001
BD+EGF <sub>D</sub> vs Sham	<0.001	BD+EGF <sub>D</sub> vs Sham	NS
BD+EGF <sub>D</sub> vs BD	<0.05	BD+EGF <sub>D</sub> vs BD	<0.001
BD+GH <sub>D</sub> vs Sham	NS	BD+GH <sub>D</sub> vs Sham	NS
BD+GH <sub>D</sub> vs BD	<0.05	BD+GH <sub>D</sub> vs BD	<0.001
<b><i>Liver Growth hormone</i></b>			
<b><i>With steatotic livers grafts</i></b>		<b><i>With non-steatotic livers grafts</i></b>	
Experimental groups	P value	Experimental groups	P value
BD vs Sham	<0.05	BD vs Sham	NS
BD+EGF <sub>D</sub> vs Sham	NS	BD+EGF <sub>D</sub> vs Sham	<0.01
BD+EGF <sub>D</sub> vs BD	<0.05	BD+EGF <sub>D</sub> vs BD	<0.01
BD+GH <sub>D</sub> vs Sham	<0.001	BD+GH <sub>D</sub> vs Sham	<0.001

BD+GH <sub>D</sub> vs BD	<0.01	BD+GH <sub>D</sub> vs BD	<0.001
<b><i>Plasma Epidermal growth factor</i></b>			
<b><i>With steatotic livers grafts</i></b>		<b><i>With non-steatotic livers grafts</i></b>	
Experimental groups	P value	Experimental groups	P value
BD vs Sham	<0.001	BD vs Sham	NS
BD+EGF <sub>D</sub> vs Sham	NS	BD+EGF <sub>D</sub> vs Sham	<0.001
BD+EGF <sub>D</sub> vs BD	<0.001	BD+EGF <sub>D</sub> vs BD	<0.001
<b><i>Liver Epidermal growth factor</i></b>			
<b><i>With steatotic livers grafts</i></b>		<b><i>With non-steatotic livers grafts</i></b>	
Experimental groups	P value	Experimental groups	P value
BD vs Sham	<0.001	BD vs Sham	NS
BD+EGF <sub>D</sub> vs Sham	NS	BD+EGF <sub>D</sub> vs Sham	<0.05
BD+EGF <sub>D</sub> vs BD	<0.001	BD+EGF <sub>D</sub> vs BD	<0.01
<b>Hepatic damage in donors with steatotic liver grafts</b>			
<b><i>Plasma ALT</i></b>			
<b><i>With steatotic livers grafts</i></b>		<b><i>With non-steatotic livers grafts</i></b>	
Experimental groups	P value	Experimental groups	P value
BD vs Sham	<0.001	BD vs Sham	<0.01
BD+EGF <sub>D</sub> vs Sham	<0.01	BD+EGF <sub>D</sub> vs Sham	<0.001
BD+EGF <sub>D</sub> vs BD	<0.01	BD+EGF <sub>D</sub> vs BD	<0.01
BD+GH <sub>D</sub> vs Sham	<0.001	BD+GH <sub>D</sub> vs Sham	<0.001
BD+GH <sub>D</sub> vs BD	<0.001	BD+GH <sub>D</sub> vs BD	<0.001
BD+EGF <sub>D</sub> +GH <sub>D</sub> vs Sham	<0.001		
BD+EGF <sub>D</sub> +GH <sub>D</sub> vs BD	<0.001		
<b><i>Plasma AST</i></b>			
<b><i>With steatotic livers grafts</i></b>		<b><i>With non-steatotic livers grafts</i></b>	
Experimental groups	P value	Experimental groups	P value
BD vs Sham	<0.001	BD vs Sham	<0.01
BD+EGF <sub>D</sub> vs Sham	<0.001	BD+EGF <sub>D</sub> vs Sham	<0.001
BD+EGF <sub>D</sub> vs BD	<0.001	BD+EGF <sub>D</sub> vs BD	<0.001

BD+GH <sub>D</sub> vs Sham	<0.001	BD+GH <sub>D</sub> vs Sham	<0.001
BD+GH <sub>D</sub> vs BD	<0.001	BD+GH <sub>D</sub> vs BD	<0.001
BD+EGF <sub>D</sub> +GH <sub>D</sub> vs Sham	<0.001		
BD+EGF <sub>D</sub> +GH <sub>D</sub> vs BD	<0.001		
<b>FIGURE S2</b>			
<b>Growth hormone protein levels in recipients with steatotic or non-steatotic liver grafts</b>			
<i>Liver Growth hormone</i>			
<i>With steatotic livers grafts</i>		<i>With non-steatotic livers grafts</i>	
Experimental groups	P value	Experimental groups	P value
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.05	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>R</sub> +LT vs BD+LT	NS	BD+EGF <sub>R</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>R</sub> +LT vs BD+EGF <sub>D</sub> +LT	<0.01	BD+EGF <sub>R</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS
BD+EGF <sub>DR</sub> +LT vs BD+LT	<0.05	BD+EGF <sub>DR</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>DR</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS	BD+EGF <sub>DR</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS
BD+GH <sub>D</sub> +LT vs BD+LT	<0.01	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001
BD+GH <sub>R</sub> +LT vs BD+LT	<0.05	BD+GH <sub>R</sub> +LT vs BD+LT	<0.001
BD+GH <sub>R</sub> +LT vs BD+GH <sub>D</sub> +LT	NS	BD+GH <sub>R</sub> +LT vs BD+GH <sub>D</sub> +LT	NS
BD+GH <sub>DR</sub> +LT vs BD+LT	<0.05	BD+GH <sub>DR</sub> +LT vs BD+LT	<0.001
BD+GH <sub>DR</sub> +LT vs BD+GH <sub>D</sub> +LT	NS	BD+GH <sub>DR</sub> +LT vs BD+GH <sub>D</sub> +LT	NS
<b>Epidermal Growth Factor protein levels in recipients with steatotic or non-steatotic liver grafts</b>			
<i>Liver Epidermal Growth Factor</i>			
<i>With steatotic livers grafts</i>		<i>With non-steatotic livers grafts</i>	
Experimental groups	P value	Experimental groups	P value
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.01
BD+EGF <sub>R</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>R</sub> +LT vs BD+LT	<0.01
BD+EGF <sub>R</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS	BD+EGF <sub>R</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS
BD+EGF <sub>DR</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>DR</sub> +LT vs BD+LT	<0.01
BD+EGF <sub>DR</sub> +LT vs	NS	BD+EGF <sub>DR</sub> +LT vs	NS

BD+EGF <sub>D</sub> +LT		BD+EGF <sub>D</sub> +LT	
<b>Damage in recipients with steatotic or non-steatotic liver grafts</b>			
<i>Plasma ALT</i>			
<i>With steatotic livers grafts</i>		<i>With non-steatotic livers grafts</i>	
Experimental groups	P value	Experimental groups	P value
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.05	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>R</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>R</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>R</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS	BD+EGF <sub>R</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS
BD+EGF <sub>DR</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>DR</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>DR</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS	BD+EGF <sub>DR</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS
BD+GH <sub>D</sub> +LT vs BD+LT	<0.01	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001
BD+GH <sub>R</sub> +LT vs BD+LT	<0.01	BD+GH <sub>R</sub> +LT vs BD+LT	<0.001
BD+GH <sub>R</sub> +LT vs BD+GH <sub>D</sub> +LT	NS	BD+GH <sub>R</sub> +LT vs BD+GH <sub>D</sub> +LT	NS
BD+GH <sub>DR</sub> +LT vs BD+LT	<0.01	BD+GH <sub>DR</sub> +LT vs BD+LT	<0.001
BD+GH <sub>DR</sub> +LT vs BD+GH <sub>D</sub> +LT	NS	BD+GH <sub>DR</sub> +LT vs BD+GH <sub>D</sub> +LT	NS
<i>Plasma AST</i>			
<i>With steatotic livers grafts</i>		<i>With non-steatotic livers grafts</i>	
Experimental groups	P value	Experimental groups	P value
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>R</sub> +LT vs BD+LT	NS	BD+EGF <sub>R</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>R</sub> +LT vs BD+EGF <sub>D</sub> +LT	<0.001	BD+EGF <sub>R</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS
BD+EGF <sub>DR</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>DR</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>DR</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS	BD+EGF <sub>DR</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS
BD+GH <sub>D</sub> +LT vs BD+LT	<0.001	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001
BD+GH <sub>R</sub> +LT vs BD+LT	<0.001	BD+GH <sub>R</sub> +LT vs BD+LT	<0.001
BD+GH <sub>R</sub> +LT vs BD+GH <sub>D</sub> +LT	NS	BD+GH <sub>R</sub> +LT vs BD+GH <sub>D</sub> +LT	NS
BD+GH <sub>DR</sub> +LT vs BD+LT	<0.001	BD+GH <sub>DR</sub> +LT vs BD+LT	<0.001
BD+GH <sub>DR</sub> +LT vs BD+GH <sub>D</sub> +LT	NS	BD+GH <sub>DR</sub> +LT vs BD+GH <sub>D</sub> +LT	NS



<b>Regeneration parameters in recipients with steatotic or non-steatotic liver grafts</b>			
<i>% PCNA positive-hepatocytes</i>			
<i>With steatotic livers grafts</i>		<i>With non-steatotic livers grafts</i>	
Experimental groups	P value	Experimental groups	P value
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>R</sub> +LT vs BD+LT	NS	BD+EGF <sub>R</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>R</sub> +LT vs BD+EGF <sub>D</sub> +LT	<0.001	BD+EGF <sub>R</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS
BD+EGF <sub>DR</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>DR</sub> +LT vs BD+LT	<0.001
BD+EGF <sub>DR</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS	BD+EGF <sub>DR</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS
BD+GH <sub>D</sub> +LT vs BD+LT	NS	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001
BD+GH <sub>R</sub> +LT vs BD+LT	NS	BD+GH <sub>R</sub> +LT vs BD+LT	<0.001
BD+GH <sub>R</sub> +LT vs BD+GH <sub>D</sub> +LT	NS	BD+GH <sub>R</sub> +LT vs BD+GH <sub>D</sub> +LT	NS
BD+GH <sub>DR</sub> +LT vs BD+LT	NS	BD+GH <sub>DR</sub> +LT vs BD+LT	<0.001
BD+GH <sub>DR</sub> +LT vs BD+GH <sub>D</sub> +LT	NS	BD+GH <sub>DR</sub> +LT vs BD+GH <sub>D</sub> +LT	NS
<i>Liver Cyclin D1</i>			
<i>With steatotic livers grafts</i>		<i>With non-steatotic livers grafts</i>	
Experimental groups	P value	Experimental groups	P value
BD+EGF <sub>D</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>D</sub> +LT vs BD+LT	<0.01
BD+EGF <sub>R</sub> +LT vs BD+LT	NS	BD+EGF <sub>R</sub> +LT vs BD+LT	<0.01
BD+EGF <sub>R</sub> +LT vs BD+EGF <sub>D</sub> +LT	<0.001	BD+EGF <sub>R</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS
BD+EGF <sub>DR</sub> +LT vs BD+LT	<0.001	BD+EGF <sub>DR</sub> +LT vs BD+LT	<0.01
BD+EGF <sub>DR</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS	BD+EGF <sub>DR</sub> +LT vs BD+EGF <sub>D</sub> +LT	NS
BD+GH <sub>D</sub> +LT vs BD+LT	NS	BD+GH <sub>D</sub> +LT vs BD+LT	<0.001
BD+GH <sub>R</sub> +LT vs BD+LT	NS	BD+GH <sub>R</sub> +LT vs BD+LT	<0.001
BD+GH <sub>R</sub> +LT vs BD+GH <sub>D</sub> +LT	NS	BD+GH <sub>R</sub> +LT vs BD+GH <sub>D</sub> +LT	NS
BD+GH <sub>DR</sub> +LT vs BD+LT	NS	BD+GH <sub>DR</sub> +LT vs BD+LT	<0.001
BD+GH <sub>DR</sub> +LT vs BD+GH <sub>D</sub> +LT	NS	BD+GH <sub>DR</sub> +LT vs BD+GH <sub>D</sub> +LT	NS